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(71) Applicants: THE ROCKEFELLER UNIVERSITY

[US/US]; 1230 York Avenue, New York, New York 10065-6399 (US). SEATTLE CHILDREN'S HOSPITAL DBA SEATTLE CHILDREN'S RESEARCH INSTITUTE [US/US]; 4800 Sand Point Way NE, Seattle, Washington 98105 (US).

(72) Inventors: CROSS, Frederick R.; c/o The Rockefeller

University, 1230 York Avenue, New York, New York 10065 (US). ROUT, Michael P.; c/o The Rockefeller University, 1230 York Avenue, New York, New York 10065 (US). CHAIT, Brian T.; c/o The Rockefeller University, 1230 York Avenue, New York, New York 10065 (US). AITCHISON, John D.; c/o Seattle Children's Research Institute, 4800 Sand Point Way NE, Seattle, Washington 98105 (US). FRIDY, Peter; c/o The Rockefeller University, 1230 York Avenue, New York, New York 10065 (US). KETAREN, Natalia; c/o The Rockefeller University, 1230 York Avenue, New York, New York 10065 (US). MAST, Fred; c/o Seattle Children's Research Institute, 4800 Sand Point Way NE, Seattle, Washington 98105 (US). OLIVIER, Paul; c/o Seattle Children's Research Institute, 4800 Sand Point Way NE, Seattle, Washington 98105 (US).

(74) Agent: LOPINSKI, John et al.; Hodgson Russ LLP, 140

Pearl Street, Suite 100, Buffalo, New York 14202-4040 (US).

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(54) Title: EXPANDING AND IMPROVING NANOBODY REPERTOIRES: TARGETING SARS-COV-2

(57) Abstract: Provided are anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) VHH antibodies, and methods of making and using the VHH chain antibodies.

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EXPANDING AND IMPROVING NANOBODY REPERTOIRES: TARGETING SARS-COV-2

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. provisional application 63/486,316, filed
5 February 22, 2023, the entire disclosure of which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under GM109824 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

10 The instant application contains a sequence listing which has been submitted electronically in .xml format and is hereby incorporated by reference in its entirety. Said .xml copy, created February 22, 2024, is titled "076091_00170.xml" and is 180,066 bytes in size.

BACKGROUND

The COVID-19 pandemic, caused by Severe acute respiratory syndrome coronavirus
15 2 (SARS-CoV-2), has had a profound global impact the likes of which has not been seen in more than a century. The remarkably rapid development and distribution of vaccines undoubtedly saved many millions of lives; nevertheless, at the time of writing, mortality estimates range from 10 – 20 million, with additional profound long-lasting health impacts for many survivors. The disease appears to be transitioning to an endemic phase, thus
20 presenting a serious worldwide health problem for the foreseeable future and demanding a large-scale ongoing implementation of new prophylactics and therapeutics.

Major therapeutic strategies have utilized antibodies directed against the major Spike (S) surface envelope glycoprotein of the SARS-CoV-2 virion, various fragments of which are also the immunogens for most vaccines. Spike is a homotrimer of an extensively glycosylated
25 ~200 kDa protein composed of two major domains: S1, which contains the host receptor binding domain (RBD) that targets the angiotensin-converting enzyme 2 (ACE2) surface receptor on host cells; and S2, which upon host cell binding undergoes major conformational changes to enable viral – host membrane fusion, resulting in virus entry into the cytoplasm. Thus, antibodies that target Spike, and particularly RBD – generated through vaccination or
30 exogenously introduced - have the potential to block viral binding and entry into the cells of the host. Unfortunately, the continuing emergence of new SARS-CoV-2 Variants of Concern (VoCs; Alpha, Beta, Gamma, Delta, Omicron and subvariants) presents a significant barrier

to attaining complete control of COVID-19. These VoCs usually have many Spike mutations (especially the RBD), and thus are relatively poorly neutralized by current vaccines and antibody therapies. For instance, monoclonal antibodies (mAbs) have proven to be an effective therapeutic strategy, though sensitive to emerging variants. Moreover, mAbs are
5 limited by challenges in the ease and cost of their large-scale manufacturing, distribution, and intravenous administration.

An alternative to mAbs is a particular class of single domain antibodies termed “nanobodies”. Nanobodies are “mini-antibodies”, some 1/10th the size of regular IgGs, derived from the variable domain (V_{HH}) of variant heavy chain-only IgGs (HCAbs) found in
10 camelids (*e.g.* llamas). Each nanobody molecule is constructed of a single Ig fold, consisting of four framework regions (FRs) that intersperse and orient three complementarity determining regions (CDRs) that form the nanobody paratope. These regions are similar to FRs and CDRs of conventional antibodies. As with conventional antibodies, CDR3 is formed by VDJ recombination of germline DNA; CDR1 and CDR2 come from the germline V
15 region, and all three CDRs are then subject to somatic hypermutation, with selection for improved binding affinity to antigens. In an example, a single variable domain on a heavy chain (V_{HH}) is provided.

Nanobodies have several attractive advantages over mAbs, including: fast on-rates leading to high overall affinities; characteristics of small molecules in terms of higher tissue
20 penetration and accessibility to regions of Spike not accessible to the larger mAbs or occluded by glycosylation, greatly enhancing their potential to synergize in combination, a profound advantage they have over often poorly-synergizing conventional antibodies. They can be readily engineered, including humanization to minimize immunogenicity; they are highly denaturation-resistant, giving them long shelf lives and making them suitable for a
25 broader range of delivery methods (*e.g.*, via nebulization directly into lungs); and very low cost of production in bacterial or yeast expression systems. Like mAbs, nanobody binding can be disrupted by mutations in VoCs.

In view of limitations of mAbs, and the advantages of nanobodies, a need remains for rapidly producing nanobodies that target the entirety of the Spike protein as a more effective
30 therapy against the continuing emergence of new SARS-CoV-2 VoCs. The present disclosure addresses these and related needs.

BRIEF SUMMARY

The present disclosure provides single domain antibodies that specifically recognize SARS-CoV-2 epitopes. The described antibodies are heavy chain only antibodies. The antibodies contain a single variable domain which comprises three complementarity-determining regions (CDRs). The disclosure includes all heavy chain only antibodies that contain any CDR1, CDR2, and CDR3 disclosed herein, and may have residue changes in the non-CDR segments. A representative antibody sequence is SEQ ID NO:117. The disclosure provides the described antibodies, compositions comprising the described antibodies, and methods of using the described antibodies for prophylaxis or therapy for SARS-CoV-2 infections.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

Figure 1. Design of Yeast-Based Nanobody Screen for anti-SARS-CoV-2 Spike Domains. Schematic of our yeast display based strategy for generating, identifying, and characterizing large, diverse repertoires of nanobodies that bind the spike protein of SARS-CoV-2. The highest quality nanobodies were assayed for their ability to neutralize SARS-CoV-2 pseudovirus. Figure adapted from (20); diagram of yeast display construct (bottom left) adapted from (34). Boxed inset shows 2.8 μ m magnetic beads conjugated with S1 protein from SARS-CoV-2 Spike, after a binding reaction with yeast displaying an anti-S1 nanobody (top) or a nonspecific control (bottom), after non-binding yeast were washed away. Figure 1 Sequence to determine VHH library are ATGGCTGAGGTGCAGTTGG (SEQ ID NO:172); ATGGCTCAGGTGCAGCTGG (SEQ ID NO:173); and ATGGCTGATGTGCAGTTGG (SEQ ID NO:174). Sequences to determine library of captured VHH are ATGGCTCAGGTGCAGCTGG (SEQ ID NO:175) and ATGGCCCAGGTGCAGCTGG (SEQ ID NO:176).

Figures 2A and 2B. Characterization of Competition Effects between Yeast Displaying Nanobodies of Different Defined Affinities. **Figure 2A.** Fluorescence microscopy of a yeast competition assay. Here, yeast displaying either LaG94-10 (K_d =2.9 μ M) or LaG9 (K_d =3.9 nM) anti-GFP nanobodies were live surface-labeled with either Alexa594 (red) or Alexa350 (green) (40). These were mixed together in equal proportions (50:50:0,

‘competitive’ conditions; see text) with GFP-conjugated 2.8 μm magnetic beads (small green spheres), to which they bound (left panels; see also Figure 1). Right panel shows a low magnification field of the assay after several rounds of harvesting and washes, showing in this case the enrichment of the LaG94-10 displaying yeast; scale bar = 10 μm . **Figure 2B.**

- 5 Plot of the coefficient of variation (CV) of yield for each yeast strain across the experiments in B against the K_d of their displayed nanobodies. As seen in the plot, the yields of yeast bearing low affinity nanobodies were highly variable specifically under competitive conditions. The yield of the highest-affinity Nb-bearing yeast, in contrast, were almost invariant because they always won the competition. Under non-competitive conditions, this
- 10 differential was lost.

- Figures 3A through 3C.** Sequence Diversity of Nanobody Libraries. Nanobody sequences from the unselected yeast library (from llama 7704; $\sim 1.5 \times 10^7$ independent clones) were amplified and sequenced with Illumina Miseq and processed to minimize sequence errors, as described in Methods, yielding 1.2×10^6 distinct nanobody sequences.
- 15 Framework regions (FRs) and complementarity-determining regions (CDRs) were determined in each encoded nanobody based on the alignment of (41). Unique sequences were determined and aligned by left justification of each region. **Figure 3A.** A seqlogo (44,59) was generated (MATLAB seqlogo command). High variability in the three CDR regions is evident. **Figure 3B.** Plot of proportion of non-consensus residues per residue
- 20 across the library. At each position the sequence diversity was calculated (defined as the probability that two randomly chosen sequences are identical at the position (60). High variability in CDRs (red bars) is again observed, as well as lower but significant variation in the FRs (blue bars). **Figure 3C.** Plot of sequence diversity per residue across the library (60). The library was sorted into CDR3 ‘families’: groups of unique sequences (minimum family
- 25 size 100) differing by no more than 20% in any of the seven regions (this criterion extracted about 15% of the unique sequences into 370 CDR3 families). Because this criterion results in high similarity of CDRs within a CDR3 family, it is very likely that the sequences in each CDR3 family derive from a unique VDJ recombination with subsequent somatic
- 30 hypermutation. For each CDR3 family the sequence diversity was calculated, and the aggregate average diversity graphed as in B. As expected, the diversity within CDR3 families was significantly reduced compared to the whole library (note difference in y-axis scale), and the differential between FRs and CDRs largely lost.

Figures 4A and 4B. Validation of the Yeast Display Method with Biochemically Identified Nanobodies. **Figure 4A.** Behavior of the yeast clones carrying CDRs matching the

mass spectrometry positive nanobodies (*i.e.*, ‘MS positives’) in the Spike domain affinity selection assay. The read counts in the unselected and selected libraries for all these sequences are plotted on top of the plot of the overall results from the entire llama 7704 nanobody cDNA display library (gray points), as plots of the log2 of these values + 1, as shown in Figure 9. 1× selection: one round of binding. 2×: selection: yeast from the 1× selection were grown out, expression of the nanobody fusion re-induced, and the yeast were bound to the same antigen (see Results, Methods); screens were for binding to either the S1 domain (S1 selection), RBD domain (RBD selection), or S2 domain (S2 selection) of Spike. In green are nanobodies that were shown previously to bind to S1 but not RBD; in blue are those that bound to both RBD and S1; in red are binders to S2. Note that all these nanobodies bound with precisely the specificity based on prior biochemical characterization (20) with the exception of a small number that failed to bind in any of the selections. These ‘MS positives’ generally bound well in both the 1× and 2× selections. These results support the specificity and comprehensiveness of the yeast display procedure. **Figure 4B.** Correlation of yeast display binding with the affinity of their displayed nanobodies, for the MS-positives plotted in part A. Plot of enrichment (log2(bound)-log2(unselected)) versus log10 (nanobody Kd). Top: 1× selection. Bottom: 2× selection. Pearson R values and associated P values by t-test are shown. The figure shows a noisy but significant correlation between binding affinity and enrichment by yeast display binding, especially for the more highly competitive 2× binding assay (see text).

Figures 5A through 5C. Screening for New Families of Anti-Spike Nanobodies.

Figure 5A. The read counts in the unselected and 1× and 2× selected libraries screened against either the S1, RBD or S2 domains of Spike from the entire llama 7704 nanobody cDNA display library (gray points) are plotted, as the log2 of these values + 1, as shown in Figures 4A, 4B. Sequences displaying different specificities were identified and selected from the graphical display as in Figures 4A, 4B, except here nanobodies specific for only the RBD subdomain of S1 were separately labeled from those that recognized the non-RBD portions of S1. Selection was by a differential polygon function allowing multiple criteria. For example, sequences in an enriched polygon for S1 but not for RBD defines nanobodies binding to the non-RBD subdomain of S1 (also see Introduction). Green: S1 non-RBD; blue: RBD; red: S2. The darker colors show the initial CDR families taken from the ~200 highest read counts sets; the lighter colors represent an extension of these CDR families to a broader set allowing up to 20% changes in any of the three CDR sequences, and above a minimum read count. Note that all these CDR families are bound with consistent specificity, indicating

that in general, sequence variations within the families (see Figures 3A-3C) do not strongly affect function. **Figure 5B.** To evaluate sequence diversity within these functional classes, the MATLAB command ‘phytree’ was employed to construct a neighbor-joining tree for the ~200 abundant CDR sequences in each class (*i.e.*, using the sequences identified by the dark green, blue or red points in panel A). A wide diversity of sequences was observed, which sorted out into a more limited set of CDR families of related sequences. Families with generally larger representation are labeled with Roman numerals; black dots indicate sequences that were expressed and characterized as recombinant monomers (see below); these were chosen from the tree to sample broadly across the sequence space, while minimizing repetitive sampling of families with clones already characterized from the mass-spectrometrically identified nanobodies (20). **Figure 5C.** Sequence conservation within the CDR families indicated in (B) was evaluated using the MATLAB seqlogo command (44) applied to the CDR strings of the selected CDR families; shown are SeqLogos of (left to right in each SeqLogo) CDR1, CDR2 and CDR3.

Figures 6A and 6B. Testing the Standard and Shuffled Libraries against RBD variants Delta and Omicron. **Figure 6A.** Testing the standard library against RBD variants Delta and Omicron. Dynabeads conjugated with RBD from the original SARS-CoV-2 and from the Delta and Omicron variants were employed for affinity purification of yeast display clones from the llama 7704 library. Two rounds of selection were carried out, as in Figures 4A, 4B, and 5A-5C. A clear overall reduction in binding was observed, though many clones still bound well to both variants. Plotted on top of the overall library result (gray points) are sequence families illustrating some of the main patterns of response to the variants are indicated as colored dots, with each color corresponding to the indicated family, defined here by their core CDR3 amino acid sequence. These families contain considerable sequence diversity within them (Figs. 5A-5C), and represent much larger numbers of many other unrelated clones (often with lower representation in the library) that exhibit similar behaviors. **B.** Testing a shuffled anti-RBD library for CDR autonomy and rescued activity in recombinants. (Top). Binding of the shuffled library to RBD. One round of purification was carried out, followed by sequencing. Results were analyzed based on the fate of CDR3 ‘groups’ (where within a group, a given CDR3 bound to a high diversity of CDR1,2 recombinants). Different behaviors were observed; three are illustrated with colored dots (same color scheme as Figures 7A-7C). The ‘NAAAW’ (SEQ ID NO:160) group (green) bound well to original and variant RBDs, essentially independent of the recombinant CDRs 1 and 2 it was attached to, suggesting strong ‘CDR3 dominance’ of the ‘NAAAW’ (SEQ ID

NO:160) CDR3. Similarly, the ‘IIDDY’ (SEQ ID NO:164) group exhibited essentially similar behavior when shuffled as when combined with its native CDR1,2 (Figures 7A-7C): strong binding to original and Omicron, but clearly weaker binding to Delta. The ‘YERLAWD’ (SEQ: ID NO:159) recombinant group bound comparably to all variants, in contrast to its behavior with its native CDRs 1 and 2, which rendered it unable to bind Delta or Omicron. **Figure 6B.** Effectiveness of the shuffle is shown by extracting all sequences bearing a specific CDR3 and examining sequence diversity of CDRs 1 and 2. Sequence logos show that highly diverse CDRs 1 and 2 are observed joined to each of three different CDR3’s, and the pattern of CDR1,2 diversity attached to the CDR3’s was essentially the same. The sequences on Figure 6B are YERLAWD (SEQ: ID NO:159), NAAAW (SEQ ID NO:160), and IIDDY (SEQ ID NO:164).

Figures 7A through 7C. Biophysical and Neutralization Properties of the Nanobodies. Thirty yeast display nanobodies targeting the S1-RBD, S1 non-RBD, and S2 portions of spike were functionally tested for neutralization of lentivirus pseudotyped with various SARS-CoV spikes and their biophysical properties characterized. (Red text indicates YERLAWD (SEQ: ID NO:159) family members, and blue text indicates NAAAW (SEQ ID NO:160) family members, used in the crossover ‘rescue’ studies; see text). **Figure 7A.** Neutralization data against Original, Delta and Omicron. **Figure 7B.** Kd measurements of 21 of these nanobodies were determined using SPR and affinities plotted. Nanobodies were tested against Original, Delta and Omicron recombinant S1 or RBD. S1 non-RBD nanobodies were not tested against Omicron. Kd measurements for three of the ‘rescue’ constructs plotted at right. **Figure 7C.** The Tm measurements of the nanobodies in (B) were determined using DSF and plotted. CoV2-YD-6 and CoV2-YD-38 (highlighted in gray) resulted in two distinct melting peaks. Open circles indicate less proportion of this species in the sample. Tm measurements for three of the ‘rescue’ constructs plotted at right.

Figure 8. Epitope Binning by Yeast Display. Dynabeads conjugated with RBD were blocked with monomer nanobodies representing the 7 epitope classes defined previously (20); with the soluble extracellular domain of the RBD target Ace2; or left unblocked. The 2×-RBD-selected library from llama 7704 (Figs. 4, 5, S1) was bound to these beads. The bound VHHs were sequenced, and enrichment/depletion upon blocking for each ‘CDR string’ (catenated CDR1/CDR2/CDR3) was calculated as $\log_2$ (readcount with blocked beads/readcount with unblocked beads). The sequences were filtered to remove PCR crossover artifacts (Methods). Left: The resulting matrix of ~100,000 sequences X 8 blocking agents was filtered for readcount (at least 100 reads combining all blocking experiments) and

clustered using the MATLAB hierarchical clustering algorithm; scale bar on left indicates log2 enrichment/depletion (above). Right: The location of sequences containing the indicated CDR3, or close relatives, was determined. Note that the hierarchical clustering was sequence-blind, so the clustering of related sequences implies similar blocking behavior across the family. Bottom: Representative nanobodies (yellow ribbon) binding to their respective epitopes (blue surface patch), as defined previously (20), are depicted on the receptor binding domain (RBD) of SARS-CoV-2 spike (PDB ID: 6M0J). The sequences on Figure 8 are NAAAW (SEQ ID NO:160), TALLS (SEQ ID NO:162), TADLY (SEQ ID NO:163), IIDDY (SEQ ID NO:164), TVDAQ (SEQ ID NO:165), AAHVN (SEQ ID NO:166), MATSEY (SEQ ID NO:170), GSDFGDH (SEQ ID NO:171), and TVTDR (SEQ ID NO:181).

Figure 9. Binding of nanobodies expressed in yeast to the different recombinant S1, RBD and S2 domains of SARS-Cov2 Spike protein (labeled S1, RBD, and S2 selection respectively) (20) conjugated to Dynabeads. Binding and washing were as described in Methods. Nanobody sequences were amplified and sequenced as in Figures 3A-3C. The CDR regions were extracted from each sequence, and concatenated in a ‘CDR string’. The read counts for each unique CDR string in the unselected library and the selected libraries were compared and, after standardization to reads per million nanobody sequences, plotted (gray points). Because of the wide spread of values, the inventors plot the log2 of these values + 1 (the addition of 1 allows plotting of zero reads as the value 0). For all three antigens the inventors observe two lines of points, one above the $x=y$ line and one below. Both lines trend upwards with increasing read count in the unselected library. The upper line was taken to represent specific binding (enriched; labeled with red oval as “specific binders”), and the lower line nonspecific binding (depleted; labeled “nonspecific binders”); the blue line ($x=y$) thus represents neither enrichment nor depletion. Both lines increase with a slope of one, probably simply because there are more yeast bearing that sequence due to differential abundance in the llama. The vertical displacement of the specific from the non-specific line represents the overall enrichment of specific over non-specific binding in our conditions, approximately 1000-fold. This is in agreement with the differential binding we achieved comparing yeast with a control surface nanobody to yeast with a high-affinity anti-GFP nanobody to Dynabeads bearing GFP (Figure 2). 1× selection: one round of binding. 2× selection: yeast from the 1× selection were grown out, expression of the nanobody fusion re-induced, and the yeast were bound to the same antigen (see Methods). The main effect of this was to reduce the population of the lower (nonspecific) line.

Figure 10. Validation of yeast display method with MS-positives from another llama. In (20) the inventors searched for nanobodies in two llamas, 7704 (“Rocky”) and 5094 (“Marley”). High-affinity binders were recovered from llama 5094, though apparently fewer than from 7704, reflecting the results of (20). The inventors carried out the same assay for these nanobodies and plotted the results as in Figure 4A, with the exception that to get sufficient representation CDR strings with up to 20% sequence variation in each CDR were accepted: each different color of the dots plotted represents a member of a family of a given mass spectrometry-positive nanobody. The same high specificity and recovery in yeast display for the 5094 clones was observed for the 7704 clones. Note that in contrast to 7704, 2X binding was required for a clean result in some cases; this is likely due to the overall lower anti-Spike nanobody content in 5094 noted previously (20), which increases the level of non-specific binding in the 1×.

Figures 11A and 11B. Comparison of different methods for screening the display libraries. The entire llama 7704 nanobody cDNA display library was screened against the RBD domain of Spike, and plotted as in Figure 9 (gray points). Two methods of screening were employed. The first was as described in Figure 1 and Methods, using two rounds of Dynabead selection. The second was following a published procedure with the first selection utilizing Miltenyi magnetic beads and biotin purification, which was followed by a second selection utilizing a FACS sorting protocol with Alexa-labeled RBD (34) (Methods). **Figure 11A.** All sequences recovered are plotted as in Figure 9. **Figure 11B.** Families of MS-positive sequences are plotted on top of the overall graph, as in Figure 10.

Figure 12. Testing CDR families of mass-spectrometry positives against RBD variants Delta and Omicron. The data are plotted as in Figures 7A-7C, but plotting CDR families of the mass-spectrometry-positive clones; each different color of the dots plotted represents a different mass spectrometry-positive nanobody.

Figure 13. Diagram of the splice-overlap extension method for recombining CDRs. Degenerate oligos priming in both directions from two highly conserved sequences within framework regions (FRs) 2 and 3, and end oligos tagged for recombination onto the yeast expression vector, were used to amplify fragments containing CDRs 1, 2 and 3 as well as flanking framework regions. The template used was a pool after 1 round of selection on RBD. The result of the sequential PCR reactions indicated is a random mix-and-match of the three CDRs and flanking framework regions.

Figure 14. Epitope Binning Tests. Dynabeads conjugated with RBD were pre-blocked with saturating amounts of either monomer nanobodies S1-1 or S1-23, with

unblocked Dynabeads as control. Yeast expressing the anti-RBD nanobodies S1-1, or S1-23, or S1-RBD-38 (each belonging to different epitope classes as defined by (20)), or the anti-GFP nanobody LaG94-10, were bound to these beads. After binding and washing, the recovery of yeast was quantified by colony counts of serial dilutions on yeast medium. S1-1-expressing and S1-23-expressing yeast were blocked from binding by the cognate but not the non-cognate nanobody block.

Figure 15. Crossover Epitope Binning. The sequences clustered in Figure 8 were curated computationally to eliminate a population of PCR crossover events (see Methods) - these are essentially similar to the 'shuffled' products made intentionally (Figures 6A, 6B). These crossover products were analyzed separately (left), and observed intriguing behavior in some cases. For example, while blockage by #6 and #7 was mutually exclusive in the native sequences (Figure 8), crossover products with CDR1 and 2 derived from the 'YERLAWD' (SEQ ID NO:159) native sequence and CDR3 from the 'NAAAW' (SEQ ID NO:160) native family were blocked by *both* #6 and #7; the converse recombinants (CDR1/2 from 'NAAAW' (SEQ ID NO:160) and CDR3 from 'YERLAWD' (SEQ ID NO:159)) behaved similarly (these sequences provide almost the entire signal in the boxed region in the figure). Numerous other examples of similar apparent 'hybrid' epitope classes were observed among the crossover recombinants. Biochemical analysis would be required to determine if such behavior represents a bona fide two-part epitope with CDR1/2 binding at one place and CDR3 binding at another; however, at present we cannot exclude that the 'hybrid epitope classes' are specific to the potentially multi-point attachment of polyvalent yeast to a polyvalent binding surface.

DETAILED DESCRIPTION

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Every numerical range given throughout this specification includes its upper and lower values, as well as every narrower numerical range that falls within it, as if such narrower numerical ranges were all expressly written herein.

As used in the specification and the appended claims, the singular forms "a" "and" and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another example includes from the one particular

value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent “about” it will be understood that the particular value forms another example. The term “about” in relation to a numerical value encompasses variations of +/-10%, +/- 5%, or +/- 1%.

5 This disclosure includes every amino acid sequence described herein and all nucleotide sequences encoding the amino acid sequences. Every antibody sequence and antigen binding fragments of them are included. Polynucleotide and amino acid sequences having from 80-99% similarity, inclusive, and including and all numbers and ranges of numbers there between, with the sequences provided here are included in the invention. All
10 of the amino acid sequences described herein can include amino acid substitutions, such as conservative substitutions that do not adversely affect the function of the protein that comprises the amino acid sequences.

 The present specification also provides polynucleotides that hybridize under selective hybridization conditions to a polynucleotide that encodes a described antibody. In a
15 representative example, a polynucleotide that can hybridize to a polynucleotide that encodes a described antibody has 70-100% complementarity, inclusive, and including all numbers and ranges there between, to the coding polynucleotide. Selective hybridization conditions under which a polynucleotide having at least 70% complementarity to a polynucleotide encoding a described antibody will be known by those skilled in the art. The degree of stringency can be
20 controlled by one or more of temperature, ionic strength, pH, and the presence of a denaturing agent such as formamide.

 A polynucleotide that hybridize under selective hybridization to a coding polynucleotide can be present in an expression vector. The expression vector can be introduced into a cell of a cell culture to thereby express a described antibody. The antibody
25 can be separated from the cell culture and used to produce a purified form of antibodies.

 In one aspect the disclosure provides a VHH chain of an anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nanobody, wherein the amino acid sequence of the VHH chain comprises any one of the sequences as set forth in SEQ ID NOs:117 – 153. The disclosure expands the repertoire of VHH chains disclosed in PCT
30 application no. PCT/US2021/047019, published as WO2022040603 on February 2, 2022, the entire disclosure of which is incorporated herein by reference, and beyond the constructs described in Fred D Mast, et al. (2021) Highly synergistic combinations of nanobodies that target SARS-CoV-2 and are resistant to escape eLife 10:e73027.

The disclosure provides VHH chains comprising amino acid sequences that have at least 90% sequence identity to any one of the contiguous sequences as set forth in SEQ ID NOs: 117 – 153. The nanobodies are referred to herein from time to time as “binding partners” in the plural and “binding partner” in the singular.

5 In some examples, the VHH chain can comprise an amino acid sequence comprising any one of the sequences as set forth in SEQ ID NOs: 117 – 153. In examples, a VHH chain as in any one of SEQ ID NOs: 117 – 153 may be combined with a VHH chain as in any one of SEQ ID NOs: 1-116 to produce a multi-specific binding partner. In an example, the multi-specific binding partner is provided in the form of a dimer that can bind to two different
10 epitopes of a viral spike protein. In examples, a VHH chain as in any one of SEQ ID NOs: 117 – 153 can be incorporated into, for example, a chimeric antigen receptor, single-chain Diabodies (scDBs), single-chain variable fragment (scFv), and other antibody fragments that retain antigen binding function.

 In examples, a binding partner of this disclosure may be used in combination with a
15 binding partner comprising a sequence of any one of SEQ ID NOs: 1-116. The combination may comprise at least two separate binding partners, or at least two antibodies may be combined into a single binding partner format to provide a multi-specific binding partner, such as a bi-specific antibody.

 In examples, a binding partner of this disclosure may be modified such that it is
20 present in a fusion protein. In examples, an antigen binding segment of a binding partner may be present in a fusion protein, and/or a constant region may be a component of a fusion protein. In examples, a fusion protein comprises amino acids from at least two different proteins. Fusion proteins can be produced using any of a wide variety of standard molecular biology approaches, including but not necessarily limited to expression from any suitable
25 expression vector. In examples, a binding partner described herein may be present in a fusion protein with a detectable protein, such as green fluorescent protein (GFP), enhanced GFP (eGFP), mCherry, and the like. In examples, as an alternative to an expression vector, an mRNA or chemically modified mRNA encoding any binding partner described herein can be delivered to cells such that the binding partner is translated by the cells.

30 Pharmaceutical formulations containing binding partners are included in the disclosure and can be prepared by mixing them with one or more pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers include solvents, dispersion media, isotonic agents, and the like.

In examples, an effective amount of one or more binding partners is administered to an individual in need thereof. In examples, an effective amount is an amount that reduces one or more signs or symptoms of a disease and/or reduces the severity of the disease. An effective amount may also inhibit or prevent the onset of a disease or a disease relapse. A
5 precise dosage can be selected by the individual physician in view of the patient to be treated. Dosage and administration can be adjusted to provide sufficient levels of binding partner to maintain the desired effect. Additional factors that may be taken into account include the severity and type of the disease state, age, weight, and gender of the patient, desired duration of treatment, method of administration, time and frequency of administration, drug
10 combination(s), reaction sensitivities, and/or tolerance/response to therapy. In examples, a described binding partner is administered to an individual who has or is suspected of having or is at risk of contracting a SARS-CoV-2 infection. In examples, the SARS-CoV-2 infection is by any type of SARS-CoV-2 that is a variant of high consequence, a variant of concern, a variant of interest, or a variant being monitored. In examples, any described antibody can be
15 administered for therapeutic or prophylactic purposes. In examples, one or more described antibodies can be administered to an individual who has been diagnosed with COVID-19.

Binding partners and pharmaceutical compositions comprising the binding partners can be administered to an individual in need thereof using any suitable route, examples of which include intravenous, intramuscular, intraperitoneal, subcutaneous, oral, or inhalation
20 routes.

The compositions may be introduced as a single administration or as multiple administrations or may be introduced in a continuous manner over a period of time. For example, the administration(s) can be a pre-specified number of administrations or daily, weekly, or monthly administrations, which may be continuous or intermittent, as may be
25 therapeutically indicated.

In some examples, the VHH chain can comprise an amino acid sequence comprising any one of the sequences as set forth in SEQ ID Nos: 117-120, 147, and 148. In some examples, SEQ ID NOs: 117-120, 147, and 148 can be selective for binding to non-Receptor Binding Domain (RBD) of SARS-CoV-2 spike protein.

30 In another aspect, the disclosure provides for an immunoconjugate comprising: (a) a VHH chain as recited above; and (b) a conjugating part comprising a detectable marker, a drug, a radionuclide, or an enzyme, or a toxin.

In some examples, the VHH chain can comprise an amino acid sequence comprising at least 90% sequence identity to any one of the sequences as set forth in SEQ ID NOs: 150-

157. In some examples, SEQ ID NOs: 150-157 are selective for binding to Receptor Binding Domain (RBD) of SARS-CoV-2 spike protein.

In some examples, the VHH chain can comprise an amino acid sequence comprising at least 90% sequence identity to any one of the sequences as set forth in SEQ ID NOs: 117-120, 147, and 148. In some examples, SEQ ID NOs: 117-120, 147, and 148 are selective for binding to non-Receptor Binding Domain (RBD) of SARS-CoV-2 spike protein.

In another aspect, the disclosure provides for an immunoconjugate comprising: (a) a VHH chain as described above; and (b) a conjugating part comprising a detectable marker, a drug, a radionuclide, or an enzyme. Such constructs may be used for therapeutic or diagnostic purposes.

In another aspect, the disclosure provides for a pharmaceutical composition comprising: (a) a VHH chain as above or an immunoconjugate as described above; and (b) a pharmaceutically acceptable carrier.

As used herein, the terms “nanobody”, “nanobodies”, “SARS-CoV-2 nanobody”, or “SARS-CoV-2 nanobodies” are exchangeable and refer to nanobodies that specifically recognize and bind to the Spike protein of SARS-CoV-2 and to the Spike protein of any variants of concern (VoC).

As used herein, the terms “single domain antibody (VHH)” and “nanobodies” have the same meaning referring to a variable region of a heavy chain of an antibody, and construct a single domain antibody (VHH) consisting of only one heavy chain variable region. It is the smallest antigen-binding fragment with complete function.

As used herein, the term “variable” means that certain portions of the variable region in the nanobodies vary in sequences, which forms the binding and specificity of various specific antibodies to their particular antigen. However, variability is not uniformly distributed throughout the nanobody variable region. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions in the variable regions of the light and heavy chain. The more conserved part of the variable region is called the framework region (FR). The variable regions of the natural heavy and light chains each contain four FR regions, which are present in a β -folded configuration, joined by three CDRs which form a linking loop, and in some cases can form a partially β -folded structure. The CDRs in each chain are closely adjacent to the others by the FR regions and form an antigen-binding site of the nanobody with the CDRs of the other chain (*see* Kabat *et al.*, NIH Publ. No. 91-3242, Volume I, pages 647-669. (1991)). The constant regions are not directly involved in the binding of the nanobody to the antigen, but they exhibit different effects or

functions, for example, in antibody-dependent cytotoxicity of the antibodies. Thus, the described antibodies may have changes from the sequences expressly described herein, including conservative amino acid substitutions in the framework regions.

In an example, the heavy chain variable region of described nanobody comprises 3
5 complementary determining regions: CDR1, CDR2, and CDR3 having at least 95% sequence identity to a described CDR amino acid sequence.

As used herein, “sequence similarity” and “sequence identity” have their usual meaning in sequence alignment. For example, sequence similarity refers to the likeness between at least two sequences in comparison. Sequence identity refers to the number of
10 characters that match exactly between the two different sequences.

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise,” “comprising,” and the like, are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to indicate, in the sense of “including, but not limited to.”

15 Reference to sequence identity addresses the degree of similarity of two sequences, such as protein sequences. Determination of sequence identity can be readily accomplished by persons of ordinary skill in the art using accepted algorithms and/or techniques. Sequence identity is typically determined by comparing two optimally aligned sequences over a comparison window, where the portion of the peptide sequence in the comparison window
20 may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino-acid residues occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of
25 comparison and multiplying the result by 100 to yield the percentage of sequence identity. Various software driven algorithms are readily available, such as BLAST N or BLAST P to perform such comparisons.

Any described antibody may be humanized. Methods for humanization are known in the art. In an example, human framework sequences are substituted for the described
30 framework sequences, suitable human framework sequences being known in the art.

EXAMPLES

To produce the described nanobody repertoires, we employed a mass spectrometry-based approach (20,22) in which llamas were immunized with Spike constructs. This permits

affinity maturation processes *in vivo* (33). High-throughput DNA sequencing of V_HH libraries PCR-amplified from marrow lymphocyte cDNA from the immunized llamas in combination with mass spectrometric (MS) identification of high-affinity V_HH regions derived from the serum of the same animal was performed. Computational matching of MS-sequenced peptides to V_HH cDNA sequences allowed high-confidence identification of sequences encoding high-affinity nanobodies. Genes encoding nanobodies were synthesized and expressed in bacteria, and nanobodies were purified, and characterized for their specificity and affinity. These V_HH cDNA libraries also represent a resource that can be used for an orthogonal approach for nanobody production, employing display screening methods instead (34-37). This approach could discover additional nanobodies, and also serve as a platform to explore the specificity and VoC sensitivity of a large number of nanobodies in parallel. Recently, a robust and efficient yeast display method was designed and validated specifically for screening nanobodies, in particular against Spike (34,38). In that work, a synthetic nanobody library was employed. In contrast, the present disclosure includes evaluation of the cDNA library made from immunized llamas (20), which was transferred into a nanobody display vector (34). We tuned the display approach and selected large repertoires of nanobodies that were specific for different domains of Spike and contained members that displayed high affinity and resistance to VoCs, and also employed DNA shuffling of the CDRs to generate variants with novel VoC specificities. This disclosure demonstrates that the yeast display method, either on its own or in parallel with the mass spectrometric method, can generate large nanobody repertoires that may be used therapeutically or prophylactic agents against COVID-19.

Optimization of Yeast Display Screening

A non-limiting depiction of a general pipeline design in Fig. 1. We used a nanobody display vector (34), which gave efficient and selective binding of specific nanobodies to diverse targets (GFP, and the S1, RBD and S2 regions of Spike). Instead of published approaches such as rounds of fluorescence-activated cell sorting (FACS) (39), or Miltenyi magnetic bead and biotin-based purification followed by FACS (34), we employed Dynabeads coupled to antigen for all selection steps (Fig. 1, inset). For most of these experiments we generated a set of four anti-GFP nanobodies in the display vector (22) with a broad range of known affinities, testing for binding of yeast expressing these to GFP-conjugated Dynabeads, as well as an irrelevant non-specific nanobody control. We also cloned three previously characterized anti-Spike nanobodies (20) into the yeast display vector

to test binding to various Spike domain-conjugated Dynabeads. We used all these well-characterized nanobodies as benchmarks to optimize conditions in terms of ease, specificity, and yield, trying for an optimal combination of high binding of specific nanobody-expressing yeast and low binding of control yeast carrying just the vector. We tested a range of binding buffers and conditions to optimize specific binding to these beads, taking advantage of the extreme robustness of yeast to even harsh binding conditions including high salt, as well as acidic and detergent washes. We found that a buffer, related to ones used for immunofluorescence microscopy and immunoblotting, gave excellent signal-to-background with these benchmarks (see Methods). Often, for specific binding, multiple beads were attached to each yeast cell, whereas no beads would be seen bound in the nonspecific control (Figs. 1, 2A). Magnetic isolation of the Dynabead-binding yeast was found to give the highest yields while maintaining efficient removal of nonspecific yeast when the magnet was placed at a distance from the yeast washing suspension such that it took several minutes to fully harvest the beads; closer placement of the magnet led to loss of cells during the repeated washes. On each wash step the beads were fully resuspended with minimal displacement of specifically-attached yeast from them. We found that 4-5 wash steps were sufficient to achieve a 100-1000-fold enrichment of specific binders over the control in a single round of isolation, which was sufficient for clear identification of specific binders by comparison of sequence counts before and after binding (Fig. 9).

20 Determination of the Roles of Nanobody Affinity and Competition During Screening

Competition has the potential to dramatically limit the repertoire, so we evaluated conditions where we could isolate high affinity nanobodies, but not at the expense of competition among yeast - an effect that could be exacerbated by avidity effects. Therefore, we used the model nanobody display strains to determine the avidity effects of the many nanobodies displayed per yeast cell, and competition between yeast cells displaying nanobodies of differing affinities for the same antigen. In order to distinguish the cells of strains displaying nanobodies of differing affinities, we covalently stained cell walls with vital fluorescent dyes (40) (Fig. 2A). To examine the effects of competition, we mixed equal cell numbers of two strains of yeast each uniquely dyed and expressing one of two different anti-GFP nanobodies of differing affinities (22), and also unlabeled control cells expressing an irrelevant nanobody. These three cell populations were in ratios of 50:50:0 or 1:1:98 respectively, all at the same final cell density and volume; the amount of GFP-Dynabeads was also held constant. Experiments suggested that in the 50:50:0 condition, beads might be

limiting, since we observed depletion of free beads not bound to yeast in the final harvest (see Fig. 1 for an example of bead depletion). In contrast, at the 1:1:98 ratios there appeared to be abundant beads remaining after binding and washes. Therefore, the experiment carried out at 50:50:0 is termed ‘competitive’ and the 1:1:98 is termed ‘noncompetitive’.

5 To assess the effect of competition as a function of binding affinity, we quantified the variability of yield across all possible pairwise binding experiments (Fig. 2B). The yield of yeast displaying low-affinity nanobodies was highly variable, because when competing with yeast displaying successively higher affinity nanobodies the yield of the former was increasingly depleted. This effect was far stronger in the competitive versus noncompetitive
10 conditions. In contrast, yeast expressing high-affinity nanobodies displayed little variation in yield even under competitive conditions, because they ‘won’ the competition. These results clearly demonstrate that the yeast display system is sensitive to the monomer binding affinity of the displayed nanobodies. We find this result surprising due to avidity considerations, but the effect seems quite clear, and was further validated in testing anti-Spike nanobodies of
15 known affinities (see below). This effect would become exacerbated under the increasingly competitive conditions that will arise through repeated rounds of panning characteristic of display methods, leading to isolation of the most competitive clones at the expense of other less competitive but still potentially valuable high affinity clones. Thus, we instead sought to take advantage of (i) starting with a hyper-immune animal and (ii) the high signal-to-
20 background of optimized panning conditions to limit the screens to just two rounds of panning, which we found to be sufficient under these conditions to identify strong positive clones that outcompete non-specific or low affinity clones, not at the expense of outcompeting other valid high affinity clones.

Examination of the Sequence Diversity of the Yeast Display Libraries.

25 We used gap-repair in yeast to clone into the yeast display vector B-cell V_HH cDNA from two llamas immunized with Spike proteins, 5094 (‘Marley’) and 7704 (‘Rocky’) (20). We obtained libraries of respectively $\sim 5 \times 10^6$ and $\sim 1.5 \times 10^7$ independent clones. We carried out next-generation sequencing on the inserts amplified from the transformed yeast. We observed highly diverse sequences, especially in the known hypervariable regions CDR1,2,3,
30 and also considerable though lesser variation in the framework regions (Fig. 3A, B). We noted that the library contained many ‘families’ of closely related but distinct sequences. Variability within families was reduced compared to variability in the library overall, but was still substantial even in the framework regions (Fig. 3C). Positions of variation in framework regions within families were similar to the variable positions in the overall library. Without

intending to be bound by any particular theory, we believe this is likely due to these variable positions occupying loop or surface positions far from the antigen binding site in the nanobody structure, since this has been noted previously for positions of nanobody variation (41,42). This, in turn, suggests that diversity within these sequence families was generated under selection in the llama, implying that this variation is largely due to somatic hypermutation after ‘founding’ of the family by V-D-J recombination. But it remains possible that variation was artifactually introduced during cloning and sequencing. The multiple related sequences in these families effectively provide a large number of biological replicates for binding experiments, as shown below.

10 Validation of the Yeast Display Method with Biochemically Identified Nanobodies

We screened the yeast display libraries from the two Spike-immunized llamas using the protocol optimized with the control strains (above; see Methods). Dynabeads coupled to S1, RBD or S2 each bound to approximately 1% of the clones in the yeast library. To evaluate this binding reaction, we amplified and sequenced the selected clones, and compared the recovery of sequences to their representation in the unselected library shown in Fig.3. To simplify the analysis as well as to focus on the major paratope (i.e., epitope-binding) region of each nanobody, we reduced each sequence to a representation consisting solely of its three CDR regions (CDRs 1,2,3) (referred to as a ‘CDR string’). We plotted the log2 of read-count for each CDR string in the unselected library vs. the log2 of read count in the selected libraries (in all cases after correction to reads per million total sequenced). We selected over two rounds with the viral antigens coupled to Dynabeads (‘1X’ and ‘2X’). Fig. 9 shows the behavior of the entire library. We observe two broad lines of plotted points, one showing strong enrichment (ratio of selected to unselected much greater than 1, shown by the line) and one showing strong depletion (below the line). Both broad lines have slopes of ~1, indicating a first classification of sequences into specific and non-specific binders, as indicated in red in Fig. 9. The rising slope in both cases is due simply to more recovery of sequences that are more abundant in the initial library (showing why it is important to have sequences for the initial as well as the selected libraries). Similar plots were obtained for all three viral antigens (Fig. 9) and both libraries (Fig. 10).

30 Previously, used a biochemical and mass spectrometry-based method and cloned and expressed 116 high-affinity anti-Spike nanobodies (20), here termed ‘MS positives’. We therefore used the biochemically characterized nanobodies as fiduciary markers to analyze their behavior when displayed by yeast. These nanobodies bound with the expected

specificity when expressed in yeast. Similar results were obtained with both animals (Figs. 4A and 10). For 5094, to increase representation due to lower overall anti-Spike nanobody levels in this animal as described previously (20), we also plotted close relatives (no more than 20% sequence divergence in any CDR) of the MS positives. The screen clearly distinguished specifically binding and non-binding clones; for example, biochemically defined S2-specific nanobodies bound to S2 beads but not S1 or RBD beads when expressed in yeast, and vice versa. We plotted enrichment of these MS positive sequences after both 1 and 2 rounds of selection against their measured K_D s (20). While the relationship was noisy, a statistically significant negative slope was observed (Fig. 4B), especially for two rounds of selection (likely to be more competitive conditions based on our analysis of anti-GFPs above), once again confirming the competitive and affinity-sensitive nature of the screen. Collectively, these results validate the screen's ability to identify large numbers of *bona fide* high affinity Spike-binding nanobodies, as well as revealing two other key behaviors. First, members within a related family behave similarly, in terms of relative enrichment and specificity during panning (e.g., Figs. 9 and 10). Second, some families came to dominate the final panned populations, particularly after two rounds of selection; for example, clones with a CDR3 containing the sequence string "GANAAH" (SEQ ID NO:183) made up more than 90% of the recovered sequences from 5094 with RBD- or S1-Dynabeads (Fig. 10). However, overall diversity of the positive families was still preserved despite distortion of representation.

We used this same benchmark to evaluate previously described panning methods (34,38), in which after a first round of magnetic bead purification, subsequent panning rounds utilize flow cytometry after binding of fluorescent RBD (Fig. 11). We found the first step of the previously described method, with magnetic bead-based enrichment of binders, gave similar results to the presently described procedure. The second flow-cytometry based step effectively eliminated non-specific binders; however, we also noted that the broad representation observed with the described method was lost, and the recovered clones were strongly dominated by a single sequence (with identical CDRs to the MS positive S1-RBD-38 (20)). The stringent removal of non-specific binders is likely important for screening naive libraries as in (34,38), but we find that it is not required in our context starting with V_{HH} cDNA from hyperimmunized llamas, where our method clearly preserves the high diversity present in the original cDNA.

Screening for New Families of Anti-Spike Nanobodies

We next analyzed the screens described above and in Fig. 4 to discover novel nanobodies not previously identified by the mass spectrometric method, focusing mainly on the more diverse repertoire of animal 7704 (20,43). We selected for analysis, using a polygon function (see Methods), the ~200 most abundant and highly enriched clones from the first round of antigen selection specifically for that given antigen, and the CDR families for those ~200 clones were also selected (dark and light colors respectively; Fig. 5A, upper row). These were found to comprise the most highly enriched clones from the second round of antigen selection, and preserved their antigen specificities (Fig. 5A, lower row).

We examined the sequence diversity and content of these positive classes by examination of neighbor-joining trees (Fig. 5B). While a selection of sequences were similar or identical to the previously described nanobodies by the MS-based approach (20), we also observed many new sequences. We used CDR3 as a benchmark for defining a given family, because its generation by VDJ recombination is the unique clonal event founding a large cluster of related sequences by somatic hypermutation. The estimates for the number of families that recognize each spike domain vary depending on the sequence parameters used to define each family, the antigen in question, the cutoff for enrichment used during a panning round, the cutoff for minimum read count (to exclude spurious sequencing / PCR errors), and the minimum size of a family. We used similarities within CDR3 to define families, with each family being defined as being made of members related to each other within a certain value or less of sequence identity. For example, for animal 7704 with the more inclusive parameters of 70% or more CDR3 sequence identity and a minimum enrichment of twofold, we found 259 S2-specific families, 90 RBD-specific families also recognizing S1, and 60 S1 (non-RBD) families. There are a further 106 families, mostly low readcount, that recognize RBD but not S1, presumably antigens buried in the full Spike protein. It is clear that a very large number of anti-Spike families - often over one hundred per domain - can be identified by this approach.

We constructed a neighbor joining tree, and though not a 'phylogeny' tree in the strictest sense, the nodes of the tree nevertheless correspond to the sequence families discussed above. Major representative families are denoted in Fig. 5B with a Roman numeral and Seqlogos (44) were constructed for these families (Fig. 5C). It can be seen that the CDR1,2,3 sequences are very diverse between families, but largely contain only minor variations within families. Moreover, a given family shows absolute antigen specificity, such that *e.g.* an RBD-specific family is not found enriching in an S2 screen. The S1-non-RBD

clones are dominated by members of one family, defined by its ‘IAQY’ (SEQ ID NO:185) consensus CDR3 sequence (Fig. 5B,C; family (i)). This family was missed in the MS-based approach (20), possibly because the CDR3 was too small for reliable peptide identification. However, numerous other new S1-specific families are present, for example the

5 “RGLGRGLGFY” (SEQ ID NO:186) CDR3 consensus sequence (Fig 5B, family (ii)). By contrast, and in agreement with its antigenic nature (12,20,45,46), the RBD domain isolated a large and diverse set of families. One of the largest families (Fig 5B, family (v)) contains the consensus CDR3 “TVDAQSDY” (SEQ ID NO:184), which is also found in the mass spectrometrically identified nanobody S1-RBD-38. Two large families identified here contain

10 divergent relatives among the MS-identified nanobodies (20), the “‘LRSRFNAAAWTTEAAFDY’ ” (SEQ ID NO:158) (previous MS-identified S1-RBD-6 and S1-RBD-31) and ‘YERLAWDTSTY’ (SEQ ID NO:177) families (previous MS-identified S1-RBD-35), the remaining four indicated families being completely novel. The S2-specific clones were also very diverse and not dominated by any single family (Fig. 5B,C), with

15 limited overlap with the mass spectrometrically identified clones (20). Collectively, the screening method identified a large number of new clones recognizing different Spike domains. We then analyzed if these new clones had high affinity binding and strong antiviral activity when expressed as monomers.

Testing the Library against the Major VoCs Delta and Omicron

20 One of the greatest challenges to managing COVID-19 is the ability of the SARS-CoV-2 virus to mutate into new VoCs that can resist prevalent vaccines and therapeutics. An advantage of generating large repertoires of nanobodies is that one maximizes the likelihood of finding VoC-resistant, broadly specific nanobodies (20,47-49). At the time of writing, two variants, and derivatives thereof, remain of significant concern, Delta and Omicron. We

25 wanted to evaluate the utility of yeast display for analyzing the sensitivity of our nanobody repertoire to these variants, focusing on the RBD domain as it is the region of largest mutational variation (50,51), and the 7704 animal as it has the largest representation. We conjugated recombinant Delta and Omicron RBD to Dynabeads and tested for binding to the yeast library compared to the original SARS-CoV-2 strain’s RBD (two rounds of selection,

30 with sequence analysis after each). Remarkably, overall, we observed that most sequences bound well to both variants (Fig. 6A; Fig. 7). For example, the large ‘TVDAQSDY’ (SEQ ID NO:184) (Fig. 5C, (v)) family binds comparably to the original SARS-CoV-2 and both variants. The “‘LRSRFNAAAWTTEAAFDY’ (SEQ ID NO:158) ‘NAAAW’ (SEQ ID

NO:160) (Fig. 5C, (iv)) family binds comparably to the original SARS-CoV-2 and the Delta VoC RBDs, and appears collectively slightly weaker against the Omicron RBD. The ‘YERLAWDTSTY’ (SEQ ID NO:177) (‘YERLAWD’) YERLAWD (SEQ ID NO:159) (Fig. 5C, (iii)) family binds well to the original SARS-CoV-2 but binding is essentially eliminated to both variants. The ‘IIDDYGVQY’ (SEQ ID NO:178) (‘IIDDY’ IIDDY (SEQ ID NO:164)) (Fig. 5C, (vi)) family (Fig. 6A, ‘IIDDY’ (SEQ ID NO:164)) and a family not indicated on Fig. 5 but comprising a family characterized by a ‘TADLYSDY’ (SEQ ID NO:179) (‘TADLY’) (SEQ ID NO:163) CDR3 sequence binds well to original SARS-CoV-2 and the Omicron variant but more weakly to the Delta variant, especially after two rounds of selection. These families contain considerable sequence diversity within them (Fig. 5). There are many other unrelated clones (often with lower representation in the library) that exhibit similar behaviors (Fig. 6A), greatly expanding the useful repertoire. A similar behavior was seen for the ‘MS positives’ in our screening (Fig. 12). This screening method is therefore potentially a rapid and straightforward way to further characterize the library for the VoC-specific sensitivities of the positive clones.

Testing the Modularity of CDRs by DNA Shuffling

DNA shuffling is an established *in vitro* method for improvement of binding or catalytic activity. Typically it is applied to a library of randomly point-mutagenized sequences that have been selected for improved activity, from which starting point splice-overlap-extension (SOE) PCR is carried out to produce mix-and-match recombinants. Two advantages ensue from this method compared to simple clonal descent by cycles of mutagenesis and selection. First, deleterious mutations hitchhiking with selected mutations are readily crossed away; second, combinations of positive mutations in different regions can be combined in a single jump through sequence space that might be highly unlikely to occur by single-mutation steps. Improved activity, and even new biological activities entirely lacking in the starting material, can potentially be found in the products. It is interesting that while point mutagenesis is a biological strategy naturally used in the process of somatic hypermutation to improve antibody affinity, DNA shuffling is much rarer in natural biological systems. VDJ recombination shares some features, but with the critical difference that only a single round of shuffling occurs rather than multiple rounds interleaved with the recombinations. Neither the extremely high density of recombination joins that can be attained by *in vitro* shuffling, and its highly multiparental nature, are shared by natural biological systems, to our knowledge.

DNA shuffling has been applied to nanobodies with recombination between CDRs, with improvement of binding noted in the progeny (52-54). However, the specificity and number of potential parental sequences was not established. We started with the library selected on SARS-Cov2 RBD, carried out SOE recombining CDRs 1, 2 and 3 at random from that library (Fig. 13). We bound the recombinant library (containing about 10⁶ members) to RBD-Dynabeads, and carried out next-generation sequencing on both the input shuffled library and the selected sequences. This allowed us to quantitatively evaluate the degree of enrichment over the shuffled unselected library.

We expected that incompatibility of CDRs from entirely unrelated nanobodies would result in a large majority of the shuffled progeny having highly reduced binding activity; however, this was not observed. A high proportion of the shuffled library could bind to RBD-Dynabeads at least to some extent. We organized the data by first examining the fate of specific CDR3 sequences, examining what CDR1 and CDR2 sequences were associated with high or low binding to RBD (either the original sequence (henceforth here termed Original), or the Delta and Omicron variants, as described already for the initial library (Fig. 6A)). In general, different CDR3 groups retained similar overall specificity of binding to Delta and Omicron to what was seen in the corresponding native families (Fig. 6). However, a notable exception was observed with the 'YERLAWD' (SEQ: ID NO:159) CDR3. The native YERLAWD (SEQ: ID NO:159) family bound efficiently to Original but not to Delta or Omicron RBD. The shuffled YERLAWD (SEQ: ID NO:159) group, in contrast, contained abundant members that bound equally well to Original and to Delta RBD, while remaining almost completely defective in Omicron binding. Examination of the sequences associated with this high Delta binding revealed specific enrichment of sequences highly similar to the native 'NAAAW' (SEQ ID NO:160) CDR1 and CDR2, recombined with the 'YERLAWD' (SEQ: ID NO:159) CDR3 (Fig. 6B). One explanation of this observation could be that the native 'NAAAW' (SEQ ID NO:160) family CDR1 and CDR2 have specific ability to bind to Delta RBD, independent of CDR3 content. However, direct examination of all shuffled products containing these CDR1,2 sequences shows that many fail to bind Delta RBD; binding requires specific CDR3s as well. So, fusion of CDR1,2 from the native NAAAW (SEQ ID NO:160) family to the YERLAWD CDR3 may create a fusion with effective multipoint attachment to the Delta RBD.

We biochemically characterized this apparent 'rescue' of 'YERLAWD' (SEQ: ID NO:159) binding to Delta RBD by expressing four distinct 'NAAAW' (SEQ ID NO:160) native parents and two 'YERLAWD' (SEQ: ID NO:159) native parents, and recombinants

between them, as monomeric recombinant nanobodies (Fig. 7B; see also next section). As expected from the yeast display data (Fig. 6), the two ‘YERLAWD’ (SEQ ID NO:159) parent nanobodies failed to bind Delta RBD while binding strongly to the Original RBD; and, the four ‘NAAAW’ (SEQ ID NO:160) parent nanobodies bound strongly to RBD from both strains (Fig. 7B). However, these hybrid nanobodies bound less well to Original and Delta RBD than either of their parents (with approximately equal affinities to Original and Delta), failed to neutralize (not shown), and showed denaturation at relatively low temperatures (Fig. 7C). These defects could simply reflect minor folding incompatibilities in the shuffled construct. Nevertheless, the shuffling results overall suggest significant modularity in CDR function, and could provide a novel avenue to new specificities which could be useful in diverse contexts.

Neutralization and Biophysical Characterization of the Isolated Nanobodies on VoCs

We selected 30 abundant sequences that represented a cross-section of the major families (Fig. 5; Supplementary Table 1), most of which were distinct from clones previously isolated (20). We used the bacterially expressed and purified monomers to determine binding affinities and neutralization capability, using assays we have employed previously (20); we also tested binding affinities and neutralization against Delta and Omicron variants (Fig. 7).

With regards to neutralization, of the 21 S1-targeting nanobodies tested (both RBD and non-RBD binding), all 21 were capable of neutralizing Original, while 10 were still able to neutralize both Delta and Omicron, although with reduced activity against the variants in some cases. We also tested 9 anti-S2 nanobodies for neutralization of Original; some neutralization activity was observed, although less efficient than for the better of the anti-S1 nanobodies. Such reduced neutralization was also true of our previously characterized anti-S2 nanobodies (20). A subset of the anti-S1 nanobodies were tested for binding affinity against recombinant S1 or RBD from either Original, Delta or Omicron (S1 non-RBD nanobodies were not tested against omicron) (Fig. 7B); all bound strongly to Original, displaying affinities in the nM - pM range. Two of these failed to bind only Delta, two failed to bind only Omicron and CoV2-YD-33 and CoV2-YD-34 failed to bind both variants. Aside from CoV2-YD-10, all were in agreement with their failure to neutralize Delta, omicron or both strains, and some had a moderate to strong reduced binding to Delta and Omicron, again correlating approximately with their reduced neutralization for that strain (Fig. 7A). CoV2-YD-10 neutralizes yet shows no binding to the RBD of omicron using SPR. It is possible the binding site of the nanobody may be slightly truncated in the RBD construct used for SPR

resulting in the no binding result. Lastly, all showed a moderate to strong degree of thermal stability, typical of nanobodies (Fig. 7C) (21).

Overall, the nanobody binding in the yeast display screening (Fig. 6) correlates reasonably well with that seen in the biochemical assay of the corresponding expressed nanobody (Fig. 7B). Thus, the differential affinities of the ‘YERLAWD’ (SEQ ID NO:159) and ‘NAAAW’ (SEQ ID NO:160) nanobodies for Delta RBD in both yeast display screening (Fig. 6) and in the biochemical assay of the corresponding expressed nanobody (Fig. 7B) agree, as discussed in the previous section. ‘LAYVT’ (SEQ ID NO:161) (CoV2-YD-7) shows no significant loss of affinity for either Delta or Omicron RBD in both yeast display screening and in the biochemical assay of the corresponding expressed nanobody. ‘TALLS’ (SEQ ID NO:162) (CoV2-YD-6) show partial loss of affinity for Delta RBD while retaining Omicron affinity in both yeast display screening and in the biochemical assay of the corresponding expressed nanobody. ‘TADLY’ (SEQ ID NO:163) (CoV-YD-9) shows complete loss of affinity for Delta RBD in both yeast display screening and in the biochemical assay of the corresponding expressed nanobody, while again retaining Omicron affinity in both assays. One nanobody seems something of an exception: ‘IIDDY’ (SEQ ID NO:164) (CoV2-YD-8) has lost binding for Omicron RBD in the biochemical assay but no obvious reduction in the yeast display screening; perhaps the avidity effect of the display method compensates for the loss of affinity of the monomeric nanobody (20). For the bulk of the new nanobodies tested, the magnitudes of their binding affinities, neutralization potential, and thermal stabilities were comparable to the nanobodies characterized in (20).

Epitope Mapping of the Nanobody Repertoire

Another important characterization of any nanobody repertoire is to determine the different epitopes being recognized by each nanobody, as in the case of anti-SARS-CoV-2 Spike nanobodies, exploration of a larger epitope space increases the likelihood of discovering variant resistant, strongly neutralizing nanobodies (20). This is usually done by ‘epitope binning’: finding classes of nanobodies that reciprocally inhibit each others’ binding due to competition for the same epitope. Epitope binning is generally carried out by one-on-one competitions between pairs of nanobodies; therefore, the number of assays scales with the square of the number of nanobodies to test. It occurred to us that we could use the yeast display assay to determine nanobody sequences in a given epitope bin across the entire library in a single binding experiment. We tested this idea in a preliminary experiment in which we saturated RBD-Dynabeads with three different nanobodies, each previously shown

to bind to a distinct epitope (20). We then tested those beads for binding to yeast cultures, each expressing a single nanobody in a known biochemically defined epitope bin. Prior nanobody binding quantitatively blocked binding of yeast expressing nanobodies in the same epitope bin, but had essentially no effect on binding of yeast expressing nanobodies known to be in different epitope bins (Fig. 14), thus validating the approach.

To carry out parallel epitope binning for the entire yeast display library, we followed conditions from this preliminary experiment: RBD-Dynabeads were saturated with 7 different nanobodies, each determined to represent 7 epitope classes using an integrative mapping approach which incorporated epitope binning by competitive binding, then subdivided the biochemical epitope bins based on escape mutant and crosslinking-mass spectrometry data (20) (we will refer to these subdivided bins as ‘epitope classes’). As with biochemically defined epitope bins, the classes may overlap on the RBD surface. We also attempted to block with the soluble extracellular domain from ACE2. The blocked beads were used to select binders from a library of 2-times-selected RBD binders (Figs. 4, 5). V_HH sequences from the bound population were determined, and the read counts of the sequences bound to RBD beads blocked with each nanobody were determined. The read count recovered from the blocked beads was divided by the read count from the unblocked beads, and the resulting ratios hierarchically clustered (Fig. 8). These 7 epitope classes were selected to collectively encompass essentially all of the available RBD surface (20). Consistent with this, the majority of nanobodies in our population are inhibited by blocking the RBD beads with at least one of the seven nanobody classes (Fig. 8). A minority of nanobodies were not so inhibited and may represent new epitope class(es).

Many nanobodies fall into more than one epitope class, as defined by this assay. Thus, most nanobodies in class #1 are also in class #2, and vice versa; and a similar mutuality is seen between classes #3 and #4, which in turn contains a smaller subgroup that is also found in class #5 (Fig. 8). The positions of the ‘founding’ epitopes for these classes was estimated previously from MS cross-linking data and escape mutants (20). Examination of the estimated position of these epitopes on RBD indeed indicates that there is significant overlap or adjacency between #1 and #2, and between #3, #4 (and even #5 or #6), consistent with steric clashes that could lead to the class overlaps observed (Fig. 8) (20).

We checked the fidelity of this method by examining behaviors of nanobodies previously identified and classified (20). In total, that work placed 17 nanobodies in one of these seven epitope classes (20). Of those 17, 11 were found in the library; of those 11, 9 exhibited the expected result of specific prevention of binding to RBD beads blocked with the

nanobody defining the epitope class. The remaining 2 were less clear, with a related sequence found to one (S1-RBD-21) showing some inhibition in epitope class #6 though previously assigned to #4, and the other (S1-RBD-23) showing no inhibition though previously assigned to class #5.

5 We further dissected nanobody binding behaviors in this assay by once again following particular nanobody families as defined by their CDRs (as above; Figs. 5, 6). The sequence variants within families generally fell together on the clustergram, which was generated sequence-blind, based solely on the binding behavior in the 7 blocked populations. This result supports the similar behavior of almost all the sequence variants assigned to the families. For example, essentially all of the sequences in the ‘YERLAWD’ (SEQ ID NO:159) CDR3 family were specifically blocked by #6, and essentially all of the sequences in the ‘NAAAW’ (SEQ ID NO:160) and ‘LAYVT’ (SEQ ID NO:161) CDR3 families (Figs. 5, 6) were specifically blocked by #7 (Fig. 8). Many other smaller CDR3 families also displayed similar behaviors. Interestingly, classes #1 to #4 did not singly block any significant CDR3 families, but rather acted to block them in different combinations. Thus, many of the CDR3 families were blocked by both #3 and #4, of note being the large CDR3 family characterized by the starting sequence TVDAQ (SEQ ID NO:165); however, there appears to be a range of behaviors in this large class (Fig. 8). So, some CDR3 families are essentially exclusively blocked by #3 and #4, such as those CDR3 sequences characterized by CDR3s starting with ARDD (SEQ ID NO:180), ARNQ (SEQ ID NO:81), and WRYF (SEQ ID NO:182). A number of families are similarly strongly blocked by #3 and #4, but also partially blocked (to lesser or greater extents) by #5 (Fig. 8); these include the large TVDAQ (SEQ ID NO:165), TALLS, (SEQ ID NO:162) and AAHVN (SEQ ID NO:166) CDR3 starting sequence families (Fig. 8; see also Figs. 5, 6). Then there are those nanobody CDR3 families almost equally strongly blocked by #3, #4 and #5, including S1-RBD-43 and related nanobodies (characterized by the CDR3 starting sequence AGHV (SEQ ID NO:167)). Lastly, there are nanobodies that are blocked relatively equally by classes #3, #4, and #6, notably those with CDR3 sequences starting with VDLAP (SEQ ID NO:168) or ASKTT (SEQ ID NO:169) (Fig. 8). Thus, the epitope classifications are not absolute: there are varying degrees of relative inhibition for each nanobody compared with its neighbors on the plot that indicate there are a very large number of discrete though frequently overlapping epitopes recognized by the population. In contrast, epitope classes that were deduced to be far apart on the RBD surface, such as #6 and #7, exhibit little or no overlap of depletion (intriguingly, analysis of crossover recombinants breaks this rule (Figure 15) for unknown reasons). Within these data

are results that also serve to highlight new families not discussed above, some with similar epitope class behaviors as the more common families, and also many that show new behaviors - and among them are also MS-identified with previously unknown specificity, such as close relatives of S1-RBD-16, which is blocked by both #5 and #7 epitope classes.

- 5 These behaviors suggest that the population of nanobodies effectively ‘scans’ the available epitope space.

CONCLUSIONS

We show that the version of a yeast display method presented here is capable of generating a large repertoire of high affinity nanobodies.

- 10 We also show three further adaptations of this method. The first is its use in combination with our previous biochemical / mass spectrometric methods (20,22). We show that in this way, one can significantly increase the total nanobody repertoire. Alternatively, we propose that one can adopt a “two factor” identification approach - only selecting nanobodies that are positive in both the biochemical / mass spectrometric and yeast display
- 15 methods, these being virtually certain to be true positives with high affinities. Large repertoires are advantageous to maximize epitope space, affinity, the likelihood of obtaining a desired biological activity such as viral neutralization, and candidates for advantageous synergistic mixture or oligomers of nanobodies. The second is the ability to shuffle between different versions of each CDR, which may in certain cases generate nanobodies with new
- 20 binding behaviors, and allows identification of CDR modularity across the library. The third is that the method can be adapted to allow massively parallel epitope binning, a step for generating fully characterized nanobodies, including with diagnostic or therapeutic potential. The disclosure provides new nanobodies with strong neutralization activity that may used for treatments for the continuing fight against COVID-19 (20).

25 METHODS

Library construction

- Starting with B cell cDNA from the same immunized llamas described previously (20), we amplified V_HH sequences with oligos providing flanking homology for cloning into the yeast display vector (34). We carried out gap-repair using a high-efficiency yeast
- 30 transformation method (55), which in our hands yielded a maximum efficiency of colony recovery of $\sim 1.5 \times 10^7$ colonies. We used a diploid trp1- W303 strain as recipient, selecting on ScMin-2% glucose. We experienced sporadic culture contamination problems with

environmental fungi; we found that use of canavanine (ScMin+can+lys) controlled this problem to a manageable level due to the *can1* canavanine-resistance mutation in W303. For control experiments we subcloned anti-GFP Nbs (22), also the anti-RBD nanobodies S1-1 and S1-23 (20), using standard cloning methods.

5 Surface nanobody induction

Stationary phase yeast were diluted 1:5 into 0.2% glucose-6%galactose in Sc-Min, grown overnight 30°C with rotation; supplemented with 10% volume fresh Sc-Min and an additional 3% galactose for another 24 hrs. These conditions gave strong induction of displayed nanobodies as detected using anti-GFP controls included in all experiments, so that we could detect surface nanobody by labeling cells with purified GFP. As described previously (56) we noted a substantial population of cells negative for GFP binding; plating experiments strongly suggested that these were due to plasmid loss events somehow induced by galactose incubation. Since these cells were plasmid-free they did not contribute to any downstream steps (outgrowth in selective medium or subsequent amplification of plasmid sequences), and therefore were inconsequential.

Yeast Affinity Capture

All antigens were conjugated to Thermo-Fisher Scientific (Waltham, MA) Dynabeads (M-270 epoxy 14301), following the manufacturer's protocol, with minor adaptations (57,58); GFP was made in-house (22) and the appropriate His-tagged SARS-CoV-2 spike antigens were obtained from Sino Biologicals (Chesterbrook, PA): Spike RBD, S1, or S2 ECD (original strain); Spike RBD L452, T478K (Delta); and B.1.1.529 (Omicron) Spike RBD. Yeast were vitally fluorescently labeled on their cell surfaces as previously described (40). We used GFP-Dynabeads and yeast expressing surface anti-GFP to establish conditions for binding and washing. The optimal binding buffer we discovered is described below. A 1 hr binding of yeast to beads with rotation at 30°C was followed by 4-5 washes with purification of bead-bound cells on a magnet using a Dynal MPC-6 magnetic stand, with samples kept at 2 cm from the magnet, 5 min binding per wash. All yeast affinity captures were performed in 1% BSA (Fraction V, protease-free; GoldBio (St. Louis, MO)), 1x PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄ pH to 7.4 with HCl / NaOH), 1% Tween-20 (Sigma Aldrich (St. Louis, MO)). For the epitope binning, affinity capture and subsequent library generation of the yeast was performed as described above, except that each 10 µl aliquot of the RBD-conjugated Dynabeads were pre-blocked with the

addition of 20 µg of the appropriate nanobody or Ace2 in 1% BSA, 1 x PBS, 0.1% Tween-20 rotating for 1 hr at room temperature. Affinity capture with Miltenyi beads and subsequent fluorescence-activated cell sorting (FACS) were performed as described (34).

Sequencing of nanobody clones in the purified yeast library

- 5 After binding, beads with bound cells were transferred to ScMin-2% glucose and grown out for 14-48 hrs. Cells were pelleted, lysed with Zymolyase and DNA purified on Qiagen miniprep columns following manufacturer's procedures. The DNA prep was amplified with sequencing primers and sequenced at the Rockefeller Genomics facility using an Illumina MiSeq, PE250 (early experiments), PE300 (most experiments; better sequence
10 quality due to longer overlap between the paired reads).

Nanobody cloning, expression and characterization

Cloning, expression and purification of the nanobodies, Surface Plasmon Resonance (SPR), Differential Scanning Fluorimetry (DSF) and SARS-CoV-2 Pseudovirus Neutralization Assays were performed as described (20).

15 Computational methods

- Nanobody sequences were obtained by paired-end sequencing (300 bp readlength) using Illumina MiSeq. Since each nanobody sequence was potentially represented by exactly one pair of reads, it was important to filter the data for quality. The computation was as follows: for positions covered only by one of the two paired-end reads, the quality score for
20 that position was the one assigned by MiSeq. For positions covered by both of the paired-end reads (i.e., both strands sequenced), the base call was that for the higher-quality-scored position, and the final score was the sum of quality scores for the two reads if the base call was the same, and the higher minus the lower score if the base call was different. The overall nominal probability of having no error anywhere in the sequence was then computed as $1 -$
25 $\text{Product}(-Q/10)$, product taken over all positions in the sequence, where Q is the final quality score at each position, and a cutoff of 0.9 applied. In addition, a similar calculation was applied to sequences approximately encoding CDR1, CDR2 and CDR3, with a cutoff of 0.95.

- The CDR sequences were extracted from the complete nanobody sequence using the consensus FR region sequences from (41). Their consensus sequences were used to generate
30 multiple alternate FR regions (usually with one or two substitutions each) and the best alignment to each FR (testing separately all of the candidate FR regions) was found. Sequences between FRs were assigned as CDRs 1,2,3. Subsequent computations were done

using the 'CDR string' composed of the catenated CDR1,2,3 sequences. Due to minor FR variability there were approximately 1/3 as many CDR strings as full nanobody sequences. The data indicated strong concordance among nanobodies with the same CDR string, consistent with the known primacy of CDR sequence for binding specificity (see

5 Introduction).

For all libraries, the number of sequences (nanobody or CDR string) was standardized to the total size of the library. Readcount was then adjusted to reads per million. The result was a table with rows corresponding to sequence and columns to standardized read count in a series of libraries. We created a custom viewer to compare readcount in various categories
10 (e.g., in specified polygons in a 2-D graph; containing some CDR3 sequence; etc). The viewer employed $\log_2(\text{standardized readcount} + 1)$; thus zero reads plotted at 0, 1 read at 1; higher readcounts plotted at approximately $\log_2(\text{readcount})$.

Crossover sequences (almost surely derived by PCR template-switching within the highly homologous FR regions) were identified as follows. Sequences (CDR strings) were
15 ranked in order of abundance. The most abundant initiated a list of 'native' (non-crossover sequences). Subsequent (decreasing abundance) sequences were then examined for a good match in some CDRs to a sequence in the 'native' list combined with a bad match in other CDRs. Such cases were assigned to a list of 'crossover' sequences; others (either distinct in all three CDRs, or similar in all three CDRs, to members of the native list) were appended to
20 the native list.

All computations were carried out by MATLAB code, available upon request. Sequence logos and phylogenetic trees were calculated using built-in functions in the MATLAB Bioinformatics toolbox.

For the blocking experiment we used the standardized readcounts to calculate
25 depletion/enrichment, based on $\# \text{reads in blocked library} / \# \text{reads in unblocked library}$. This was done after filtering out likely crossover products (see above).

Table 1 – nanobody sequences – CDRs are shown in italics. The disclosure includes antibodies with each set of CDR1, CDR2, and CDR3, wherein variation of amino acid
30 sequences before, between, and after each CDR sequence is permitted, such as by way of conservative amino acid substitutions. Each CDR described herein is considered to be a distinct sequence.

Table 1

Nanobody ID	Sequence	SEQ ID NO
S1-1	MAQVQLVESGGGLVQAGGSLRLSCAASGMPFSSYAMGWFRQAPGKEREFVAAV NWNIGISTYLADSVKGRFTASRDNAKNTAYLQMNSLKPEDTAVYYCAADSKGWSE WGMDYWGKGTQVTVS	1
S1-2	MAQTQLEESGGGLVQAGGSLRLSCAASGSIIIRPNVMAWYRQGPQKQRELVASIHS SGTKNYADSVKGRFTISRDNANTVYLQMNSLKPEDTAVYYCATRSTWATQPDY WGQGTQVTVS	2
S1-3	MGQVQLVESGGGLVQAGGSLRLSCAASGSTFSNTGLGWYRQAPGKQREWVASIS PSGSTSYLDSVRGRFISRDNAKNIAYLQMNSLKPEDTAVYYCNTLPRSGSWGQGT QVTVS	3
S1-4	MAQVQLVESGGGLVQAGGSLRLSCAAGRTFSSYFMAWFRQAPGKEREFVAAV DWSGGDTLYTDSVKGRFTISRDNAGANTVYLQMNNLKREDTAVYYCAADRGYSLYY YQAREYEWGQGTQVTVS	4
S1-5	MAQVQLVESGGGLVQPGGSLRLSCAASGFTLGYYAIGWFRQAPGKEREGVSCIGS SDGSTYYADSVKGRFTISRDNANTVYLQINSLKPEDTAVYYCATKGPPQGVLRCSIS GRYDFWGGQGTQVTVS	5
S1-6	MAQVQLVESGGGLVQAGDSLRLSCVVSGRSSFFSMAWFRQAPGKEREFVAVIR GSGGVTFYSESVKGRFTISRDNANTVSLQMSSLKPEDTAVYYCAGSDFGDHGPLS GRTDFSSWGQGTQVTVS	6
S1-7	MAQVQLAESGGGLVQVGDSLRLSCAASGRITYTSSMYGMAWFRQAPGKEREFVA VIGWNTYYADPVKGRFTISRDNANTVYLQMNNLKPEDTAVYYCASRKAAGLASG YPSPSYYDYWGQGTQVTVS	7
S1-9	MAQVRLVESGGGLVQPGGSLRLSCVVSQVSGSAFRFNRLGWYRQAPGKERELVA SISGGSTNYADSVKGRFTISRDNASTVSLQMNSLKPEDTAVYYCNIRADFFSSTRT GDWGQGTQVTVS	8
S1-10	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSSAGMAWFRQAPGKEREFVATS NWNGGDTDYADSVKGRFTISRDNANTVYLQMNNLKPEDTAVYYCAAHLQKYG GDYYARIWGQGTQVTVS	9
S1-11	MAEVQLVESGGGLVQFGGSLRLSCAASGFTLSPYDIGWFRQAPGKEREGVSCISVT GGTTDYTDSVKGRFTISRDHARNTVYLQMNNLKPEDTAVYYCAAHTASFCRGDYS ASVARYWGQGTQVTVS	10
S1-12	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMGWFRQAPGKEREFVVAIS WKSGLITAA NFLEGRFTISRDNANTMYLQMNNLKPEDAAVYYCAVDPNRRRG LTYYDHWGQGTQVTVS	11
S1-14	MAQVQLVESGGGLVQAGGSLRLSCAASGSTFSRLSMGWYRQVPGKQRELVARIL PLGGPYRDFVQGRFTISRDNVKNMLYLQMNSLKPEDTAVYYCNRAPFGTAWDG PDNYDYWGQGTQVTVS	12
S1-17	MAQVQLVESGGGLVQAGGSLRLSCPASGLASTYSAGWFRQAPGKEREFVAAIN RSGGITSYADSVKGRFTISRDNAKNTVYLQMNNLKPEDTAVYYCAAKVTLSDDTYN QPGNYPSWGQGTQVTVS	13

S1-19	MAQVQLVESGGGLVRAGGSLRLSCAASVSTFSSYAMGWYRQAPGNQRELVAGIS PDGSTNYADSVKGRFTISRDNANTLVLMNSLKSSEDVAVYYCRIFLPEIPGRGSW GQGTQVTVS	14
S1-20	MAQVQLVESGGGLVQPGGSLRLSCAVSGFPLDFYAIGWFRQASGKEREWVSCISR RDDYTSYVDSVKGRFTISRDNANTVYLQMNLSLKLEDAVYYCAGVVRTSSGTVCQA YPRWYDTWGQGTQVTVS	15
S1-21	MAQVQLVESGGGLVQAGGSLRLSCAVSGI AVRSTMGWYRQAPGKQRELVA TIN /GGSTNYADSVKGRFTIARDNAENTAYLQMNLSLKPEDAVYYCAQYYTG DYWG RGTQVTVS	16
S1-23	MAQVQLVESGGGLVQAGGSLRLSCVASGRTSRPYRMGWFRQAPGKEREFVAAIF WSGSHTLYEDSVKGRFTISTDNAQNTVYLQMNLSLKPEDAVYYCAARNQATGNY DVWGQGTQVTVS	17
S1-24	MAQVQPVESGGGLVQAGGSLRLSCVASGSTTTNYHMGWYRQTPGEQRELVA AI NAGGITNYADSVKGRFTISRDNANTMYLQMNNLRFEDAVYYCNI GGGWDYRN SYYIPRVDSWGQGTQVTVS	18
S1-25	MAQVRLVESGGGLVQPGGSLRLSCVSVSGVSGSFRFNRLGWYRQAPGKERELVA SISGGSTNYADSVKGRFTISRDNANKNSVSLQMNLSLKPEDAVYYCNIRADFFTSTR TGDWGQGTQVTVS	19
S1-27	MAQVQLVESGGGLAQPGGSLRLSCAASAGTFGSSRMGWFRQAPGKEREFVAAI MPSGSHTNYADSVKGRFTISRDNAKDTIYLQMNLSLKPEDAVYYCGARDPSSYTYD RWGQGTQVTVS	20
S1-28	MAQVQLVESGGGLVQAGGSLKLSCAVSGRTLSSYVMGWFRQAPGKEREFVAAIR WNGGSTFYADSVQGRLTISRDNANKNMVYLQMDSLKPEDTAAYYCAASPRTMYYA SYYT RTSYDYWGQGTQVTVS	21
S1-29	MAQVQLVESGGGQAQAGDSRLSCAASGDTFSSAPMGWFRQAPGKERELIAGIS WSALNTYYADSVKGRFTISREDAKNTVYLQMNLSLKQEDAVYHCAARTRSHFYSE VYRRASDYDFWGQGTQVTVS	22
S1-30	MAQVPLVESGGGLVQAGGSLRLSCTTSGSTFSTSHMGWYRQAPGKQRELVASIST EGTTEYADFVKGRFTISRNNNAQNTVYLEMASLKPEDAVYHCNRGGWRYYG DGS HYIPDFRFRWGQGTQVTVS	23
S1-31	MAQVQLVESGGGLVQPGGSLRLSCAASGSISIIYRGWFRQVPGKEGEFVASIDTA GSTTYADSVKGRFTISRDNANTLYLQMNLSLKPEDAVYYCKYVTWAPRVEYRGQ GTQVTVS	24
S1-32	MAQVQLVESGGGLVQAGGSLRLSCTASGVAFSLYHMAWFRQAPGKEREFVTA IN WNGGIADYVDSVKGRFTISRDNANTVYLQMNLSLKPEDAVYYCAA KL GAYYTTY RKANEYQYWGQGTQVTVS	25
S1-35	MAQVHLIESGGGLVQAGGSLRLSCVASGRTFSSLVMAWFRQAPGKEREFVAFIE WSGAETYYTDSVKGRFTISRDNANKNTVILQMNSVKPEDTG VYYCAADKGYS LYYYR ESDYEWGQGTQVTVS	26
S1-36	MAQVQLVESGGGLVQAGGSLRVSCAASERILSSYVMGWFRQAPGKEREFVAAIR WNGGSTFYADSVKGRLTISRDNANKNTVYLQMNLSLKPEDAVYYCAASPRTMYLAS YYYHRTSYDYWGQGTQVTVS	27

S1-37	MAQVQLVESGGGLVQPGGSLRLSCVVS ~~GFPLDFFA~~ IGWFRQASGKEREWVSCISR RDDYTSYVDSVNGRFTISRDN ~~AE~~ NTVYLQMNSLKLED ~~TAVYYCAGVRTSSD~~ TVCQ NYPRWYLTWGQGTQVTVS	28
S1-38	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSYAIMGWFRQAPGKDEFV ~~GW~~ MAWSGPAKYYADSVKGRFSISRDN ~~AK~~ NTVYLQMNSLKPED ~~TAVYYCAASKEIT~~ TM TTRYDYWGQGTQVTVS	29
S1-39	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFGSYAMGWFRQAPGKERECVSAI NWSGDHTYYADSVKGRFTISRDN ~~AK~~ NTVYLQMNSLKPED ~~TAVYYCAARGP~~ NEW GLPREDLFYDYWGQGTQVTVS	30
S1-41	MAQVPLVESGGGLVQAGGSLRLSCTTSGSTFSTYHMGWYRQAPGKQRELVA ~~AIN~~ SEGSTYYPDSVKGRFTISRDN ~~AQ~~ NTVYLEMASLKPED ~~TAVYHCNRGGW~~ RYYG ~~DGS~~ RYIPDFRSWGQGTQVTVS	31
S1-46	MAQVQLVESGGGLVQAGGSLRVSCVISGHQFSDYVIGWFRQAPGKEREFVGAIW WSDDITYYEDSVKGRFTVSRDN ~~AK~~ NTVYLQMNSLKPED ~~TAVYHCAVRFRPV~~ STYQ VRSTDDYGYWGQGTQVTVS	32
S1-48	MAQVQLVESGGGLVQPGGSLRLSCAASGITLDYYAIGWFRQAPGKEREGVSLISS DGSTYYADSVKGRFTISRDN ~~AK~~ NTVYLQMNSLKPED ~~TAVYYCATGSLTRYG~~ SSWH VPFGSRSYENWGQGTQVTVS	33
S1-49	MAQIQMQLVESGGGLVQAGGSLRLSCTASGSIFSIGAMSWYRQAPGKQRELVAQ ISSGGITNYADSVKGRFTISRDN ~~AK~~ NTVYLQMNTLKPED ~~TAVYYCAADE~~ TPWGAT RGWGQGTQVTVS	34
S1-50	MAQTQLEESGGGLVQAGGSLRLSCAASGSII ~~RPNV~~ MAWYRQGP ~~GKQREL~~ VASM HSSG ~~TK~~ NYAESVKGRFTISRDN ~~AK~~ NTVYLQMNSLKPED ~~TAVYYCATR~~ STWAGQPD YWGQGTQVTVS	35
S1-51	MAQVQLVESGGGLVQAGGSLRLSCAASGFSFSNY ~~YMW~~ FRQAPGKEREFVAAIT TWLSNTYYTDSVKDRFTISRDN ~~AK~~ KMLYLQMNSLKPED ~~TALYYCAA~~ EQSGRDWRR TSPSPYAYWGQGTQVTVS	36
S1-52	MAQVQLVESGGGLVQAGGSLRLSCAASGSIFSLNAKAWYRQAPGSQRELVARITG SGTITNYADSVKGRFTISRDN ~~VK~~ NTVTLQMNSLRPED ~~TAIYICAA~~ APGGLWTGPAY WGQGTQVTVS	37
S1-53	MAQVQLVESGGGLVQAGDSLRLSCAASGYTITNYWMAWFRQAPGKERESVATIT YGAGMKYYADSVKGRFTISRDN ~~T~~ KNTVYLQMESLKPED ~~TAVYYCRSPQR~~ PYSGFGS DDYWGQGTQVTVS	38
S1-54	MAQVQLVESGGGLVQAGGSLRLSCAASGIIFSKYAVAWYRQAPGKQRELVA ~~TINH~~ SGDTKYADSVKGRFTISRDN ~~AK~~ STLYLQMNSLKPED ~~TAVYYCNIGI~~ WIASAHSPDGR ADHWGRGTQVTVS	39
S1-55	MAQVQVVESGGGLVQAGGSLRLSCAVSGRTFGMRAMGWFRQAPGKEREFVAV ISTSGGDTSYADSVKGRFTISRDN ~~AK~~ NTVYLQMNTLKPED ~~TAVYYCAVPRIG~~ AWSR SPRDYDIWGQGTQVTVS	40
S1-56	MAQVQLVESGGGLVQPGGSLRLSCAASGSIFSIDAMGWFRQAPGKEREPVAHIR NTGTTAYADSVKGRFTISRDN ~~T~~ KNTVYLQMNSLKPED ~~TAVYYCNELK~~ DFLGGVIPL NSWGQGTQVTVS	41

S1-58	MAQVQLVESGGGLVQAGGSLRLSCAASGSTFSAYSMGWYRQAPGKQRELVA ⁴² ISGSGSTNYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCNTVGDYRYDYP ITGRNDYWGQGTQVTVS
S1-60	MAQVQLVESGGGLVQAGGSLRLSCAASGSTFSTNAMGWYRQAPGKERELVAEIT ⁴³ TDGTTTRYADSVKGRFTISRDNKNTVYLQMNSLKSEDTAVYYCNTVTRFLGGLLRP DYWGQGTQVTVS
S1-61	MAQVQLIESGGGLVQAGGSLRLSCAASGRTFSSYAMGWLQAPGEEREFVAAISR ⁴⁴ RGMDDTYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCARKMVAPIFVVV TTRDEYDYWGQGTQVTVS
S1-62	MAQVQLVESGGGLVQAGGSLRLSCAPSGRTSNTYPMWFRQAPGKEREFVAAIT ⁴⁵ RSVPNTYYADSVKGRFTISRDIKNTMYLQMNSLKPEDTAVYYCAGSSNVYLLTRD EYDYWGQGTQVTVS
S1-63	MAQVQLVESGGGLVQFGGSLRLSCAASGIIFSNAMGWYRQAPGKERESVALIMN ⁴⁶ NGNTYYENSVKGRFSISRDNKNTVYLQMDSLKPEDTAVYYCNAVAYRDYDRKRY DYWGQGTQVTVS
S1-64	MAQVQLVESGGGLVQPGGSLRLSCAASGSIFSVNSMGWYRQAPGKERELVATITS ⁴⁷ HGITNYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYFCHREDTGLWNSKDT YWGQGTQVTVS
S1-65	MAQVQLVESGGGLVQPGGSLRLSCAASGSIFNVNSMGWYRQAPGKERELVATIT ⁴⁸ TRGITNYADSVKGRFTISRGNKNTVHLQMNSLKPEDTAVYYCNREDTGLWNSKD VYWGQGTQVTVS
S1-66	MAHVQLVESGGGLVQAGGSLRLSCVASTRTFMGWFRQAPGKERAFVAAISWNG ⁴⁹ VSITYADSVAGRFTISRDNKNTVLLQMNSLKPEDTAVYYCAAADVAGTRWTDY NYWGQGTQVTVS
S1-RBD-3	MAEVQLVESGGGLVQAGGSLRLSCAASGFTLDTTAIGWFRQAPGKEREGVSCISTS ⁵⁰ GERTYYADSVKGRFTISRDNKNTMYLQMNSLNKPEDTAVYSCAAKYASFQYLPVG LPWSMYDVRGQGTQVTVS
S1-RBD-4	MAEVQLVESGGGLVQAGGSLRLSCAASEFTLGYYAIGWFRQAPGKEREGVSFISRH ⁵¹ GSTTYTYSVRGRFTISRDNSENTVYLQMDGLKPEDTAVYYCAASKTTAWDTIRVS AIEYDYWGQGTQVTVS
S1-RBD-5	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSSLVMGWFRQAPGKEREFVAFIE ⁵² WNGGDTYYTDAVKGRFTISRDNKNTVFLQMNSLKPEDTAVYYCAADRAYSLEY RVSEYQYWGQGTQVTVS
S1-RBD-6	MGQVQVAESGGGLVQAGDSLRLSCAASGRTFTTNAMGWFRQAPGKEREFVAAI ⁵³ SWSTGTTYSDPVKGRFTISRDNKNTVYLQMNSLKPGDTAVYYCTLRSAFNAYA WTTTERAYDYWGQGTQVTVS
S1-RBD-9	MAQVQLVESGGGSVQAGGSLRLSCAASARTFSNYVVAWFRQAPGKEREFVALIT ⁵⁴ WSGADRYYGDSVKGRFTISRDDAKNTVYLQMNSLKPEDTADYYCAADRGSYHYA ESKNYDYWGQGTQVTVS
S1-RBD-10	MAQVQLVESGGGLVQAGGSLRLSCAVSGRTFRNYAMGWFRQAPGKEREFVAVI ⁵⁵ TATDDITYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCAADYGDSSYYT RRTEYGYWGQGTQVTVS

S1-RBD-11	MAQVQVVESSGGLVQAGGSLRLSCVASGSTDSNYVMGWYRQTAGKQREWVAS INSGGETRSVDSVKGRFTISGDNAKNTVYLQMNSLKPEDTAVYYCFYERLAWDPST SWGQGTQVTVS	56
S1-RBD-12	MAQVQLVESGGGLVQPGGSLRLSCAASGSILSINDMGWYRQAPGKERELVAFITS GGSTNYAESVKGRFTVSRDNAKHAVYLQMNSLKPEDTAVYYCNADIRESGFVYGM NAWGKYWGKGLTVTVS	57
S1-RBD-14	MAQVQLVESGGGLVQAGGSVRLSCAASGRSFSINPMGWFRQAPGKEREFVAAIS WSGSKTVVVDVSVKGRFSISRDNANTVYLQMNSLEPEDTAVYHCAVASRGPVYGA NYVPGPHEYEYWGQGTQVTVS	58
S1-RBD-15	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVASIN WNGGNTYYADSVKGRFTISRDNANTVYLQMNSLKPEDTAVYYCAATGPNEYGL PREDLFYDYWGQGTQVTVS	59
S1-RBD-16	MAQVQLVESGGGLVQSGGSLRLSCVASGRGFDRYPMGWFRQAPGKEREFVGGI NWWGANTYYADSVKGRFTISRDNKNTVYLQMNSLKPEDTALYYCAADQHYMG PPPKEYEYDYWGQGTQVTVS	60
S1-RBD-18	MAQVQLVESGGGLVQAGGSLRLSCTASGITFSGYVMAWYRQAPGKQRELVGWIS HGGIKDYADSVKGRFTISRDNPKNTVYLQMNSLKPEDTAVYYCNRKDAQSSWGQ GTQVTVS	61
S1-RBD-19	MAEVQLVESGGGLVQPGGSLRLSCVASGFSFSSYGMGWFRQAPGKEREFVATISG TGGNTAYSDSVKDRFTISRDNANTVYLEMNSLKPEDTAVYYCAAPPSWYTRRYIN FAPSEVRHWGQGTQVTVS	62
S1-RBD-20	MAQVQLVESGGGLVQAGDSRRLSCAVSGRTFSSGAMAWFRQAPGEERDFVAVI RWSGSTYYADSVKDRFTISTDNVKNVFLQMNNLAPEDTAVYYCAATDRTHYDIV EYTRDHFYSNWWGQGTQVTVS	63
S1-RBD-21	MAQVQLVESGGGSVQAGGSLRLSCAASGRAFSDYGMGWFRQAPGKEREFVATI DSTGSNTYYADSVKGRFTISRVAENTVYLQMNSLKPEDTAVYYCAADRFGGGRM EYRYEYWGQGTQVTVS	64
S1-RBD-22	MAQVQLAESGGGLVQAGDSLRLSCVASGRTFSAYTLAWFRLAPGKEREFVAHID WSGTETNYADSNKGRFTISRDNALNTVYLQMTGLKPEDTAVYYCAADRAYSIMYY DTREYEYWGQGTQVTVS	65
S1-RBD-23	MAQVHLVESGGDLVQPGGSLRLSCVASGSGFENNAITWYRQAPGKERELVSGITS GGSTNYADSVKGRFTISRDNANTVYLEMNSLKPEDTAVYLCQAVAWDSRRRSVV AFWGQGTQVTVS	66
S1-RBD-24	MAQVQLVESGGGLVQAGGSLRLSCVVSNGTHFSLTMAWFRQAPGKEREFVAGT DWSGINYADSVKGRFTISRDTNTLFLQMNSLKPEDTAVYYCAFDRAASLYPLRD QADYMYWGQGTQVTVS	67
S1-RBD-25	MAEVQLVESGGGLVQPGGSLKLSAASGFTLDYYTIGWFRQAPGKEREGVSYISSS GSNTYYEDSVKGRFTISRDNASTVYLQMNSLKPEDTAVYYCAARRVWQETIVVR GPEHYEYWGQGTQVTVS	68
S1-RBD-26	MAQVQLVESGGGLVQPGGSLRLSCAISGRTFGGYGVGWFRQAPGKEREFVAGITS TSFGTSYADAVKGRFTISRDNANTVTLMNSLKFEDTAVYYCAASVLSPPPRNTS AYAYWGQGIQVTVS	69

S1-RBD-27	MAQVHVVESSGGGLVQAGGSLRLSCAASGRTFGIYNMGWFRQAPGKEREVAAIT GDASDTYYADSVKGRFTISRQNAKNTVFLQMDNLKPEDTAVYYCAATGAITRATM WKPNFWGQGTQVTVS	70
S1-RBD-28	MAQVQLAESGGGLVQAGGSLRLSCAASGRTISTDAMGWFRQAPGKEREVVAISW RSDDTYYADNVKGRFTMSRDNAKNTVYLQMNLLKPEDTAVYYCVADQGRSYYT RESEYNYWGQGTQVTVS	71
S1-RBD-29	MAQVQLVESGGGLVQDGGSLRLSCAASGLASIVFNMGWFRQAPGKEREVAGIL GSGQSTYYGNSVKGRFTISRDSAKNTVYLQMNSLKPEDTAVYYCAAGGRDLVNTT GGYEPTYWGQGTQVTVS	72
S1-RBD-30	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSSLVMGWFRQAPGKEREVAFIE WSGAETYYTDSVKGRFTISRDNKNTVFLQMNSLKPEDTAVYYCAADAAYS�YYR VSEYEWGQGTQVTVS	73
S1-RBD-32	MAEVQLVESGGGLVQPGGSLKLSAASGFTLDYYTIGWFRQAPGKEREGVSYISS GSNSYYVDSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCAARRVWQPTIVVSG PEHYDYWGQGTQVTVS	74
S1-RBD-34	MAQVQLVESGGGLVQAGDSLRLSCVASGRTFSTTFMGWFRQAPGKERDFVAAIN WNGGSTRYADSVKGRFTISRDNKNTVYLQNSLKPEDTAVYYCAADPSDGRVV GYNYVPVSTHEYDYWGQGTQVTVS	75
S1-RBD-35	MTQVQVVESSGGGLVQAGGSLRLSCVASGSTDSDNYVMGWYRQASGKQREWVAS INSGGDTKSVDSVKGRFTISIDNAKNTMYLQMNSLKPEDTAVYYCFYEKLAWDPST TWGQGTQVTVS	76
S1-RBD-36	MAQVQLVESGGGLVQAGDSLRLSCVASGRTFSTTFMGWFRQAPGKEREVAAIN WSGGSTRYADSVKGRFTISRDNKNTVYLQNSLKPEDTAVYYCAADPSDGRVFG VNYVPVSTHEYDYWGQGTQVTVS	77
S1-RBD-37	MAQVQLVESGGGLVQAGGSLRLSCGASGDSFSFRWYGMNWYRQGPQPREWV AGISSAGSTNYADSVKGRFTISRDNKNTVYLQMNLLKPEDTAVYYCYSATQERDY WGQGTQVTVS	78
S1-RBD-38	MAQVQLVESGGGLVQAGGSLRLSCAASGSTDNYPTWYRQAPGKQREFVASIR SGHITNYANSVKGRFTMSKDNKNTVYLEMNNLLKPEDTAVYYCYTVDAQSDYWG QGTQVTVS	79
S1-RBD-39	MAQVQLVESGGGRVQAGGSLRLSCAVSGSTFSSDVMAWYRQPPGKQREWVAS FSSSGSNGYAASVKGRFTISRENAKNTVYLQMNSLKPEDTGVYYCHAAHVNGGDS WGQGTQVTVS	80
S1-RBD-40	MAQVQLVESGGGLVQAGGSLRLSCAASGITFSNYGMVWWRQAPGKQREFVASI SSGRDTNYADFKGRFTISRDNKNTVHLQMNSLKPEDTAVYYCYTVDRSDYWG QGTQVTVS	81
S1-RBD-41	MAQVQLVESGGGLVQAGGSLRLSCAASGITFSRAVGWHRQAPGKPREWVAAS NSGTTSYTDPMKGRVTISRDNKNTVYLQMINLLKPEDTAVYYCYIADGLLGDDYW GQGTQVTVS	82
S1-RBD-43	MAQVQLVESGGGLVQAGGSLRLSCAASGTFSSDVITWYRQPPGKQREWVASIQ SSGSRNYAASVKGRFTISRENAKNTVYLQMNSLKPEDTGVYYCHAGHVTGGDSW GQGTQVTVS	83

S1-RBD-44	MAQVQLVESGGGLVQAGGSLRLSCAASSSTVSNSPMDWFRQAPGKQREFVATV QSGGNASYSYSVKGRFTISRDNAAKMMVYLQMNSLKPEDTAVYYCHAAADDRSDYW GQGTQVTVS	84
S1-RBD-45	MAQVQLVESGGGLVQAGGSLRLSCLASGITWMPYGMWYRQAPGKQRFVATI SSNGRTNYAASVMGRFTISRDNANTVYLQMNSLKPEDTAVYYCHGATALSDYW GQGTQVTVS	85
S1-RBD-46	MAEVQLVESGGGLVQAGGSLRLSCAASESTLGSYAIGWFRQAPGKEREGVSFISRD GSTTFYTSSVRGRFTISRDNNTKNMVYLMQMDGLKPEDTAVYYCAASKITAWDTIIVSA IEITYWGQGTQVTVS	86
S1-RBD-47	MAQVQLVESGGGLAQTGGSRLSCAASGISFSTYDMNWFRQTPGKQQEWVAGI GTLNSPRYANAVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCNTASDRDYWG QGTQVTVS	87
S1-RBD-48	MAQVQLVESGGGWVQAGGSLRLSCSASGRTFDNYNIGFIEWFRQAPGKERTFVA TISRSGDITNYAGSVAGRFTISRDNNTVYLQMNSLKPEDTAVYYCHMSMTTGG DYWGQGTQVTVS	88
S1-RBD-49	MAQVQLVESGGGLVQAGGSLRLSCTSSASTFSIGAMGWFRQAPGKQREFVATITS SGTTSYAGSVKGRFTISRDNKNSVYLMNTLKPEDTAVYYCYANNLHGGDYWG QGTQVTVS	89
S1-RBD-51	MAQVQLVESGGGLVQAGGSLRLSCVASGRTLSTYRMGWFRQAPGKERKFVAAIF WSGGHTVYEDSVKGRFTISTDNAQNTVYLQMNSLKPEDTAAYYCAARNPATGNY DYWGQGTQITVS	90
S2-1	MAQVQVVEGGGLVQAGGSLRLSCAASTLVLGGYDMRWYRQVPNGRELVASI SNGGSTDYADFKGRFTISSDVAKNTVYLQMDSLKSEDVAVYYCNMRSTYYGIDY WGRGTQVTVS	91
S2-2	MAQVRLVESGGGLAQAGGSLRLSCAASGLQFSRITMAWYRQAPGKQRELVALTS TRGTTNYPDSVKGRFTISRDNANMNVYLQMNSLKPEDTAVYYCNTGPGYKTDYW GQGTQVTVS	92
S2-3	MAQILVTQSGGGLVQTGGSLRLSCAASTLVLGGYDMRWYRQAPGKERELVASITN GGDTRYVDSVKGRFTISSDNAKNAVYLMDSLKSEDVAVYYCNMHSTYYGRDFW GQGTQVTVS	93
S2-4	MTQVQLVESGGGLVQAGDSLRLSCVSSEPTFSSRTMGWFRQAPGKERDENVATIP WWRGALPYYKDSVKGRFTISRDSAKNTMYLQMNSLKPEDTAVYYCAASKTWSPS TGVATEYFRGQGTQVTVS	94
S2-5	MAQVQLVESGGGLVQAGGSLRLSCGASGLTFKNYAMGWFRQPPGKEREFVATIN SIGGPANYANSVKGRFTISRDNANTVYLQMNSLKPEDTAVYYCAAERTPSTLLAG SSNPSRVDVWGQGTQVTVS	95
S2-6	MTQVQLVESGGGLVQAGGSLRLSCAASAGIFGGSTMGWYRQAPGEQREMVATI SNSGSANYADSVKGRFTISRDHAKRTVYLQMDSLKPEDTAVYYCNKHSIYYGRDY WGQGTQVTVS	96
S2-7	MTQVQLVESGGGLVQAGDSLRLSCVASEPTFSSRTMGWFRQAPGKERESVAAIP WWKGALPYYKDSVKGRFTISRDSAKNTMYLQMNSLKPEDTAVYYCAASKTWSPY TDAATELEFRGQGTQVTVS	97

S2-9	MLQVQLVESGGGLVQAGGSLRLSCAASGSTFSTYGVAWYRQAPGKQRELVASINS WGWINYADSVKGRFTISRDNANKNTVSLQMNSLKPEDTAVYTCNSQNSRGDNYW GQGTQVTVS	98
S2-10	MAQVQLVESGGGLVQTGGSLRLSCAASGRIVSSAMSWFRQAPGKEREFAVIAIS SNSHTYYADAVKGRFTISRDNANKNTVYLQMNSLKPEDTAIYYCAARSHLTIAAWER LKYDDWGQGTQVTVS	99
S2-11	MAQVQLVESGGGLVQPGGSLRLSCAASGRTFSGATMGWFRQAPGKEREFAVAIL WRYEYTNANSVKGRFTISADKAKNTVYLQMNSLKPEDTAVYYCACAIERSGIYYH EDSNKYQYWGGGTQVSVS	100
S2-13	MAQVQLVESGGGLVQPGGSLRLSCAASGFTMTYYTMTWFRQAPGKEREWVSSS RITGNTYYTDSVKGRFTISRDNASTVYLQMNSLKPEDTATYYCASGRNINYYTNSA QYDYWGRGTQVTVS	101
S2-14	MAQVQLVESGGGLVQTGGSLRLSCAPSGRTFSPYAMGWFRQAPGKEREFAVIGIG WYSGTTYADSVKGRFTISSDIKNTLTLMNSLKPEDTAVYYCAASKDRYGNMDP NGVRYDYWGQGTQVTVS	102
S2-15	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSWYNMAWFRQAPGKEREFAVAGI SLTDISRSGGNTYYADSVKGRFTISRDNANKNTVYMQMDSLKPEDTAVYYCAADDR RMPAAIMTSVRDYVYRGQGTQVTVS	103
S2-18	MTQVQLVESGGGLVQAGDSLRLSCVASESTFSSRTMGWFRQAPGKERDFVAAIP WWIGALPNYKDSVKGRFTISRDSAKNTMYLQMNSLKPEDTAVYYCAASKTWSRPR TDVTELEFRGQGTQVTVS	104
S2-22	MAQVHLAESGGGLVQAGGSLRLSCAASGRTFSWYNLAWFRQTPGKEREFAVAGIG RIGGNIYYADSVKGRFTISEDNAKNTVYLKMDSLKPEDTAVYHCAADDRMPAAI MTSARDYVYWGQGTQVTVS	105
S2-26	MAQVQLVESGGGLVQAGGSLRLSCAVSGGTFSRYSMGWFRQAPGKEREFAAIS SNDKATYYKDSVKDRFTISRDNANKNTVYLMNTLKPEDTAVYYCAADPVLMKTPDI MASRYVYWGQGTQVTVS	106
S2-33	MAQVQLLESGGGLVQPGGSLRLSCVVSIGSIANMRVMKWYRQAPGKEREWVGT MFNPGPMTYRDSVKGRFTISRDNANKNTVNLQMNSLKVEDTAVYYCENVDPNRYE YWGQGTQVTVS	107
S2-35	MAQVQLVESGGGLVQPGGSLRLSCAASFRISSVEVLGWYRQTPGKERELVATLTR AGTTNYIDAVKGRFTISRDNANKNMLYLQMNSLKPEDTAVYYCENVRLIGQNFVG QGTHVTVS	108
S2-36	MAQVQLVESGGGLVQAGGSLRLSCAASGRIATNYVMDWYRQAPGKQQLVASIS NDGSTNYADSVKGRFTISRDNANKNTLYLQMNSLKPEDTAVYLCHLKHWWFGFAK YDYWGQGTQVTVS	109
S2-39	MAQVQLVESGGDLVQAGESLRLSCAASGIIISRYAMDWYRQAPGKQREFVAEISS GGTTNYADSVKGRFTIFRDNANKNTMYLQMNSLKPEDTAVYYCKVSYTTYSGQGTR VTVS	110
S2-40	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSWYNLAWFRQAPGKEREFAVAGIS RIAGNTYYADSVKGRFTISEDNAKNTVYMQMDSLKPEDTAVYHCAADDRWMPA ATMTSVRDYVYRGQGTQVTVS	111

S2-42	MAQVQLVESGGGLVQAGGSLRLSCAASGRTFSWYNLAWFRQAPGKEFEFVGGIS RTAGNTYYADSVKGRFTISEDNAKNTVYIQMDSLKPEDTAVYHCAADDRMAAAAT MTSVRDYVYRGQGTQVTVS	112
S2-47	MAQVRVEESGGDLVQAGGSLRLSCTTSAI/LNTWPMGWYRQAPGKQRELVAQ/ITS GGTTNYADSVKGRFTISRDRQNIYVLQMNRLKPEDTAVYYCNLRADRDYWGQG TQVTVS	113
S2-57	MAQVQLVESGGGLVQAGGSLRLSCVASGRIFSSYAMAWFRQAPGKEREFVATIT WSGKLTMYQDSVKGRFTISKDNPNKNTVYLQMNLTLPEDTALYYCAAHEKGWKQV SPDDVKYWGQGTQVTVS	114
S2-59	MAQVQLVESGGGQAQAGGSLRLSCAASGRTISNYNMGWFRQAPGKEREFVAAA KWSIDINESYADSVKGRFTISRDNKNTVYLMNSLKPEDTAVYYCAAAGGYSDNI RQTDYNYWGQGTQVTVS	115
S2-62	MAQVQLVESGGGLVQPGGSLRLSCAASGRTFSSAMMGWFRQAPGKEREFVASI DWRYYQYRYADSVKGRFTISRDNKNTVYLMNSLKPDDTAVYYCACQIQRDGIY YYEDSSKYQYWGQGTQVSVS	116
CoV2-YD-1	QVQLVESGGGLVQAGESLRLSCVASGITFNSFILGWYRQAPGNERELVAWIYSGG GTSYADSVRGRFTISRDDAKNTLYLQMNLSLKPEDTAIYYCNRGLGRGLGFYEGQGT QVTVS	117
CoV2-YD-2	QVQLVESGGGLVQPGGSLRLSCSISGFSFGYSAIGWFRQAPGLEREWVSTISSNGD DTVYADSVKGRFTISRDNKNTVYLMNSLKPEDTAVYYCARSFRWSRLPRDYDS WGQGTQVTVS	118
CoV2-YD-3	QVQLVESGGGLVQAGGSLRLSCVASTTWFSNFGMGWYRQAPGKQRELVAGIT DGTTKYVDSVKGRSTIARDNAKNTVSLQMNSVKPGDTAIYYCNTYPSQLWNWGQ GTQVTVS	119
CoV2-YD-4	QVQLVESGGGLVQAGGSLRLSCAASGSTFDNYPVTWYRQAPGKQREFVASIRSGH ITNYANSVKGRFTMSKDNKNTVYLEMNNLKPEDTAVYYCNIAQYWGQGTQVTV S	120
CoV2-YD-5	QVQLVESGGGLVQAGGSLRLSCVASGTTISTYNLLWFRQAPGQQREWVASISSGG DTSYAGSVKGRFTISRDNENTMYLQMNLSLKPEDTAVYYCHGANAAHGMDYWG KGTQVTVS	121
CoV2-YD-6	QVQLVESGGGSVQVGGSLRLSCAASGMTFSSATMAWYRQAPGKQREWVAGIGS GGRTSYASSMKGRFTISRDNKNTVYLMNSLKPEDTAVYYCYTALLSGGDYWGQ GTQVTVS	122
CoV2-YD-7	QVQLVESGGGLVQPGGSLRLSCTASVHPFSIYDMGWYRQAPGKQRLVASISSDG TTDYADSVKGRFTISRDNKNTVYLMNSLKPEDTAVYYCNLAYVTWGQGTQVT VS	123
CoV2-YD-8	QVQLVESGGGLVQPGGSLRLSCAASGSMNSMNPNGWYRQAPGKEREVVARISS GGSTTYTDSVKGRFTISRDSAKNTVYLMNSLKPDDTAVYYCYIIDYGVQYWGKG TQVTVS	124
CoV2-YD-9	QVQLVESGGGLVQAGGSVRLSCAASGITFSNYASQWFRQTPGKAREFVAA/ISGGG TTSYAASVKGRFTLSRDNKNTVYLMNDLKPEDTAVYYCHTADLYSDYWGQGT QVTVS	125

CoV2-YD-10	QVQLVESGGDLVQPGGSLRLSCAASGFTFSYDMGWFRQGPGRKEREFAQIAPN GITTYADSVKGRFTISRDNAMRVFLQMNSLKPEDTAVYFCAVDLAPWGSGLKRT NPARGQGTQVTVS	126
CoV2-YD-11	QVQLVESGGGLVQAGGSLRLSCVVSSTFSRYDMGWYRQAPGKARETVATISRS GTTNYAYFVKGRFTITRDNAKNTVYLQMNSLKPEDTAVYYCYIFGLDYWGQGTQV TVS	127
CoV2-YD-12	QVQLVESGGGLVQAGGSLRLSCAASGSTFSRLNLLWRQAAGKPREWVATSTDG GTRNYAAAVKGRFTISRDNAMNTVYLQMNSLKPEDTAVYYCYTPDYWGQGTQV VS	128
CoV2-YD-13	QVQLVESGGGLVQAGGSLRLSCAASGFTDDYAIGWFRQAPGKEREGVSCISSG GFTNYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAVVVNGYGLDEWAY DYWGQGTQVTVS	129
CoV2-YD-14	QVQLVESGGGLVQAGGSLRLSCSASGNTFSTYSLSWYRQAPGKQREFVAAISSGGS TSYANSVKGRFSISRDNAMKTMVYLQMNSLKPEDTAVYYCYVVTLLMDYWGQGTQ VTVS	130
CoV2-YD-15	QVQLVESGGGLVQAGGSLRLSCAASSTTFAGHTMGWYRQDPAKQRELVASITSG GTTNYADSVKGRFTISSDNAKNTVYLQMNNLKFEDTAVYYCHYHSTYYNLHWWG QGTQVTVS	131
CoV2-YD-16	QVQLVESGGGLVQAGGSLRLSCTTSVSVFVINAAGWYRQAPGKEREFVAEIASGRT TNYADSVKGRFTISTDNAGNTVYLRNLIKPEDTALYMCYIKDALNQVFWGQGTQ VTVS	132
CoV2-YD-17	QVQLVESGGGLVQAGGSLRLSCAVSGITDSTYVMGWYRQAPGKNRAMVAQITS GGVTNYAGSVKGRFTISMSRDNAMMVYLQMNSLKPEDTAVYYCKKEGARLWN DYWGQGTQVTVS	133
CoV2-YD-18	QVQLVESGGGLVQAGGSLRLSCAVSGISGSTFYTMNWYRQAPGKQRELVASIP SGAGLNYADSVKDRFAISRDNAMNTVILQMNSLKPEDTAVYYCNAYQPIWKYNW WGQGTQVTVS	134
CoV2-YD-19	QVQLVESGGGLVQAGGSLRLSCAASGRAFSMHPMAWYRQAAGKQREYVARISS GGGSTNYQDSVKGRFTISRDNAMNTVYLQMNSLKPEDTAVYYCNFGGSYSGSYYL PGDYWGQGTQVTVS	135
CoV2-YD-20	QVQLVESGGALVQAGGSLRLSCVASGRTFSGSTFRNVAMGWYRQGPGRKQREFV ARMSSGDVTNYADSVKGRFTISRDNAMNTVYLEMNSLKPEDTAVYYCNLRDLLGQ DFWGQGTQVTVS	136
CoV2-YD-21	QVQLVESGGGLVQAGGSLRLSCAASRRPWNDYLMGWYRVAPGKQRELVAVTNS AGTINYSDSVKGRFTISKDNAKNTVYLQMNSLKPEDTAVYYCYLQALPWKYEWG QGTQVTVS	137
CoV2-YD-22	QVQLVESGGGLVQAGGSLRLSCAASGSTFNGYTRGWYRQAPGKQRELVASIDSG GSTNYADSVKGRFTISRDNAMNTVYLQMNSLKPEDTAVYYCNAQWWPGTFWGQ GTQVTVS	138
CoV2-YD-23	QVQLVESGGGLVQPGGSLRLSCLAPVRILSINAMGWYRQAPGKERELVARIYRSGS TNYADSVKGRFTISRDNAMNTMYLEMNSLKPEDTAVYYCNLADSIGQDFWGQGT QVTVS	139

CoV2-YD-24	QVQLVESGGGLVQAGGSLRLSCAASG ITFSTYS MGWYRQAPGTQRELVAR ISSIGT TNYADSVKGRFTISRDN AENTV SLQMNSLKPEDTADYYCK AESER WSWQYYWGQ GTQVTVS	140
CoV2-YD-25	QVQLVESGGGLVQAGGSLRLSCAVSG ISGNTFSTYTM NWYRQAPGKQRELVA TIK SDSATSYADSVKGRFAISRDN AKNTM ILQMNTMKAEDTAVYYC NAYQPIW KYNW WGQGTQVTVS	141
CoV2-YD-26	QVQLVESGGGLVQPGGSLRLSCTTSAN VLKAN VLGWYRQTPGKERELVA TLTRAG NTNYVDSVKGRFTISRDDGKNILY LQMNS LKPEDTAVYYC NAQDLIGQ NFWGQGT QVTVS	142
CoV2-YD-27	QVQLVESGGDLVQAGGSLRLSCAASG FTLD SHHIAWFRQAPGKER EAVSC ISRLG STFYSGSVKGRFTISRDN AKNTM YLQMNSLKPEDTAVYYC AA SYRPIV TYCD LISTKY NFWGQGTQVTVS	143
CoV2-YD-28	QVQLVESGGGLVQAGGSLRLSCAASG STLNGY TRGWYRQAPGKQRELVA SISSGG TTTYADSVKGRFTISRDN AKNTV YLQMNSLKPEDTAVYYC NAQWWPG NFWGQG TQVTVS	144
CoV2-YD-29	QVQLVESGGGLVQAGGSLRLSCAAS VNSFRT LTLSWYRQASGKQRESVA SITSGGS TNYADFVKGRFTISRDN VANTV YLQMNSLKPEDTAVYYC NIRDLLA QNFWGQGT QVTVS	145
CoV2-YD-30	QVQLVESGGGLVQAGGSLRLSCAASG LTF SR YAMG WFRQAPGKEREFVA TISWD GGATYYGGSVKGRFVISRDNDKNT VHLQL NSLKPEDMAVYYC AA SR NQFY TGLTE SNYAYWGQGTQVTVS	146
CoV2-YD-31	QVQLVESGGGLVQAGKSLRLSCAASG LSFG FGKYVMGWYRQAPGNDRELVA KIY SGGGTNYADSVKGRFTISRDDVKNM LYLQM NSLKLEDTAVYYC NIAQ YYGGQGTQ VTVS	147
CoV2-YD-32	QVQLVESGGGLVQAGGSLKLS CAGSGF SERFSAYVMAWYRQAPGNDRELVA KIYS GGGTHYADSVKGRFTISRDN VKNTL YLQMINLRLED TA VYYC NIAQ YWGGQGTQVT VS	148
CoV2-YD-33	DVQLVESGGGLVQAGGSLRLSCV ASGSTD SNYVMGWHRQTSGKQSEWVA SIESG GEKKIVDSVKGRFTISRDN AKNTV YLQMNSLKPEDTAVYYC FYERLAW DTSTYWG QGTQVTVS	149
CoV2-YD-34	DVQLVESGGGLVQAGGSLRLSCV ASGSTD SNYVMGWYRQTSGKQREWVA SINS GGETRSADSEKGRFTISGD NAKNTV YLQMNSLKPEDTGVYYC FYERLAW DTSTYW GQGTQVTVS	150
CoV2-YD-35	DVQLVESGGGLVQAGDSLRLSCT ASGRFT TTNAMGWFRQAPGKEREFVA AISWS YGTYYSDPVEGRFTISRS NAKNTV YLQMNSLKPD DTA VYYCT LR SRFN AAAWTTE AAFDYWGQGTQVTVS	151
CoV2-YD-36	QVQLVESGGGLVQAGDSLRLSCT ASGRFT TTNAMGWFRQAPGKEREFVA AITWS YGTYYSDPVEGRFTISRS NAKNTV YLQMNSLKPD DTA VYYCT LR SRFN AAAWTTE AAFDYWGQGTQVTVS	152
CoV2-YD-37	DVQLVESGGGLVQAGDSLRLSCT ASGRFT TTNAMGWFRQAPGKEREFVA AISWN YDTYYSDPVEGRFTISRS NAKNTV YLQMNSLKPD DTA VYYCT LR SRFN AAAWTTE AAFDYWGQGTQVTVS	153

CoV2-YD-38	DVQLVESGGGLVQAGDSLRLSCAASGRTFTTNAMGWFRQAPGKEREFVAAISWN SGTTYSDPVKGRFTISRDNANKNTVYLQMNSLKP GDTAVYYCTLRSRFNAYAWTTE NAFDYWGGGTQVTVS	154
Rescue-1	DVQLVESGGGLVQAGDSLRLSCTASGRTFTTNAMGWFRQAPGKEREFVAAITWS YGTYYSDPVEGRFTISRDNANKNTVYLQMNSLKP EDTAVYYCFYERLAWDTSTYW GGGTQVTVS	155
Rescue-2	DVQLVESGGGLVQAGDSLRLSCTASGRTFTTNAMGWFRQAPGKEREFVAAISWN YDTYYSDPVEGRFTISRDNANKNTVYLQMNSLKP EDTAVYYCFYERLAWDTSTYW GGGTQVTVS	156
Rescue-3	DVQLVESGGGLVQAGDSLRLSCTASGRTFTTNAMGWFRQAPGKEREFVAAISWN SGTTYSDPVKGRFTISRDNANKNTVYLQMNSLKP EDTAVYYCFYERLAWDTSTYW GGGTQVTVS	157

CDR amino acid sequences described herein include the following, which may be a component of any described antibody:

- 5 LRSRFNAAAWTTEAAFDY (SEQ ID NO:158)
 YERLAWD (SEQ ID NO:159)
 NAAAW (SEQ ID NO:160)
 LAYVT (SEQ ID NO:161)
 TALLS (SEQ ID NO:162)
 10 TADLY (SEQ ID NO:163)
 IIDDY (SEQ ID NO:164)
 TVDAQ (SEQ ID NO:165)
 AAHVN (SEQ ID NO:166)
 AGHV (SEQ ID NO:167)
 15 VDLAP (SEQ ID NO:168)
 ASKTT (SEQ ID NO:169)
 MATSEY (SEQ ID NO:170)
 GSDFGDH (SEQ ID NO:171)
 YERLAWDTSTY (SEQ ID NO:177)
 20 IIDDYGVQY (SEQ ID NO:178)
 TADLYSDY (SEQ ID NO:179)

ARDD (SEQ ID NO:180)

ARNQ (SEQ ID NO:181)

WRYF (SEQ ID NO:182)

GANAAH (SEQ ID NO:183)

5 TVDAQSDY (SEQ ID NO:184)

IAQY (SEQ ID NO:185)

RGLGRGLGFY (SEQ ID NO:186)

10 Table 2 – nanobody characterization

ID	Epitope	S1			RBD		
		K _{on} (M ⁻¹ s ⁻¹)	K _{off} (s ⁻¹)	K _D (M)	K _{on} (M ⁻¹ s ⁻¹)	K _{off} (s ⁻¹)	K _D (M)
S1-1	RBD	4.14E+05	2.98E-05	7.20E-11	6.50E+05	5.98E-07	9.20E-12
S1-2	non-RBD	1.59E+06	1.88E-03	1.18E-09	No interaction detected		
S1-3	non-RBD	5.08E+05	4.32E-04	8.51E-10	No interaction detected		
S1-4	RBD	1.25E+06	1.06E-04	8.46E-11	1.26E+06	1.26E-04	1.37E-10
S1-5	RBD	1.33E+05	1.15E-03	8.61E-09	–		
S1-6	RBD	1.02E+06	5.75E-04	5.65E-10	5.92E+05	3.69E-04	6.22E-10
S1-7	non-RBD	7.59E+05	9.90E-04	1.30E-09	No interaction detected		
S1-9	non-RBD	9.51E+05	4.28E-07	4.50E-13 ^a	–		
		1.25E+05	1.57E-04	1.25E-09			
S1-10	non-RBD	8.35E+04	1.82E-03	2.19E-08	–		
S1-11	non-RBD	–			–		
S1-12	RBD	2.90E+05	8.92E-04	3.07E-09	2.33E+05	2.24E-04	9.63E-10
S1-14	RBD	1.08E+06	1.10E-03	1.02E-09	5.37E+05	7.99E-04	1.49E-09

S1-17	non-RBD	—			—		
S1-19	RBD	1.30E+06 3.55E+04	8.86E-03 2.41E-04	6.81E-09 ^a 6.81E-09	—		
S1-20	RBD	1.48E+07	4.37E-03	2.95E-10	—		
S1-21	RBD	4.77E+06	1.58E-04	3.31E-11	1.22E+06	2.45E-04	2.00E-10
S1-23	RBD	2.82E+06	4.91E-05	1.74E-11	1.09E+06	1.07E-04	9.78E-11
S1-24	non-RBD	6.49E+05	2.89E-04	4.45E-10	No interaction detected		
S1-25	non-RBD	2.15E+05	3.39E-05	1.57E-10	No interaction detected		
S1-27	RBD	3.15E+06	4.52E-04	1.43E-10	2.89E+06	6.30E-04	2.18E-10
S1-28	RBD	1.38E+06	7.97E-04	5.76E-10	1.79E+06	1.03E-03	5.77E-10
S1-29	RBD	2.39E+05	1.01E-03	4.21E-09	1.73E+05	8.89E+04	5.12E-09
S1-30	non-RBD	6.21E+05	1.48E-03	2.38E-09	No interaction detected		
S1-31	RBD	2.17E+06	5.63E-04	2.59E-10	1.94E+06	9.37E-04	4.84E-10
S1-32	non-RBD	2.73E+05	4.66E-04	1.71E-09	No interaction detected		
S1-35	RBD	2.46E+06	2.11E-05	8.60E-12	2.70E+06	9.77E-05	3.62E-11
S1-36	RBD	2.28E+06	3.92E-04	1.72E-10	7.87E+06	1.72E-03	2.18E-10
S1-37	RBD	4.03E+06	2.75E-04	6.82E-11	4.14E+06	2.09E-04	5.05E-11
S1-38	RBD	5.34E+06	1.12E-03	2.10E-10	—		
S1-39	RBD	2.14E+06	8.11E-04	3.79E-10	1.68E+06	1.06E-03	6.30E-10
S1-41	non-RBD	8.73E+05	1.38E-03	1.58E-09	No interaction detected		
S1-46	RBD	1.68E+05	2.94E-04	1.75E-09	2.22E+05	1.70E-04	7.66E-10
S1-48	RBD	2.61E+06	6.22E-05	2.39E-11	1.66E+06	1.64E-04	9.85E-11
S1-49	non-RBD	1.94E+06	3.63E-03	1.87E-09	—		

S1-50	non-RBD	3.33E+05 3.34E-03	1.39E-02 3.94E-04	4.40E-09 ^b	No interaction detected		
S1-51	RBD	9.28E+04	4.22E-04	4.54E-09	3.77E+06	2.01E-03	5.33E-10
S1-52	RBD	4.22E+05	3.13E-04	7.74E-09	4.53E+04	1.94E-04	4.36E-09
S1-53	RBD	1.40E+06 2.36E+04	8.46E-03 2.19E-04	6.05E-09 9.27E-09	–		
S1-54	RBD	1.13E+06	6.58E-05	5.84E-11	2.55E+04	2.88E-04	1.13E-08
S1-55	RBD	3.98E+06 3.53E+04	5.41E-03 5.31E-06	1.36E-09 1.51E-10	5.03E+05 1.84E+04	1.11E-02 1.82E-04	2.21E-08 9.89E-09
S1-56	RBD	1.46E+04 4.45E-03	2.99E-03 7.90E-05	3.57E-09 ^b	2.21E+03	1.05E-04	4.73E-08
S1-58	non-RBD	5.73E+05	1.66E-04	2.90E-10	–		
S1-60	non-RBD	3.30E+05 4.61E+04	5.24E-06 3.67E-03	1.59E-11 ^a 9.58E-08	–		
S1-61	RBD	9.87E+05 8.23E+02	1.81E-02 1.10E-04	1.84E-08 ^a 1.34E-07	4.46E+04	1.88E-04	4.21E-09
S1-62	RBD	2.68E+06	9.51E-05	3.54E-11	3.30E+06	6.30E-05	2.08E-11
S1-63	RBD	1.09E+06 3.39E+04	1.12E-02 1.67E-04	1.02E-08 ^a 4.94E-09	5.10E+04	2.23E-04	4.37E-09
S1-64	non-RBD	6.97E+05	1.58E-04	2.26E-10	–		
S1-65	non-RBD	1.06E+06	1.67E-04	1.57E-10	–		
S1-66	non-RBD	4.66E+05	2.74E-04	5.87E-10	–		
S1-RBD-3	RBD	8.81E-05 7.36E+04	1.76E-02 1.13E-03	2.00E-08 ^a 1.53E-08	–		
S1-RBD-4	RBD	2.02E+06	1.64E-04	8.09E-11	2.83E+06	8.16E-04	2.89E-10
S1-RBD-5	RBD	1.94E+06	1.63E-04	8.38E-11	7.21E+06	1.05E-03	1.45E-10
S1-RBD-6	RBD	1.55E+06	1.63E-04	1.05E-10	3.48E+06	1.13E-03	3.24E-10
S1-RBD-9	RBD	–			2.85E+05	1.23E-04	4.30E-10

S1-RBD-10	RBD	—			—		
S1-RBD-11	RBD	2.22E+07	2.94E-04	1.32E-11	2.06E+07	4.06E-04	1.97E-11
S1-RBD-12	RBD	—			1.10E+04	3.39E-05	3.10E-09
S1-RBD-14	RBD	—			1.33E+04	3.34E-04	2.51E-08
S1-RBD-15	RBD	5.37E+06	1.50E-04	2.79E-11	7.52E+06	4.95E-04	6.58E-11
S1-RBD-16	RBD	—			1.68E+04	6.25E-05	3.73E-09
S1-RBD-18	RBD	2.28E+06	6.25E-04	2.74E-10	4.43E+06	1.27E-03	2.87E-10
S1-RBD-19	RBD	—			—		
S1-RBD-20	RBD	2.37E+06	2.23E-04	9.43E-11	3.05E+06	7.91E-04	2.59E-10
S1-RBD-21	RBD	3.50E+06	1.31E-03	3.73E-10	3.15E+06	1.71E-03	5.45E-10
S1-RBD-22	RBD	9.34E+05	2.28E-04	2.44E-10	9.24E+05	4.42E-04	4.78E-10
S1-RBD-23	RBD	—			2.89E+06	4.61E-05	1.59E-11
S1-RBD-24	RBD	1.61E+06	1.40E-03	8.65E-10	2.12E+06	1.22E-03	5.75E-10
S1-RBD-25	RBD	—			8.41E+04	1.16E-02	1.38E-07
S1-RBD-26	RBD	1.06E+05	4.58E-06	4.32E-11	2.15E+05	1.33E-05	6.19E-11
S1-RBD-27	RBD	—			6.19E+06	1.24E-02	2.00E-09
S1-RBD-28	RBD	1.80E+06	4.27E-04	2.38E-10	1.80E+06	4.27E-04	2.38E-10
S1-RBD-29	RBD	—			5.36E+05	1.35E-03	2.51E-09
S1-RBD-30	RBD	2.15E+06	6.66E-05	3.10E-11	3.77E+06	4.82E-04	1.28E-10
S1-RBD-32	RBD	—			1.05E+05	7.90E-03	7.52E-08
S1-RBD-34	RBD	—			5.71E+04	4.88E-03	8.54E-08
S1-RBD-35	RBD	8.01E+05	1.68E-04	2.10E-10	1.33E+06	2.50E-04	1.88E-10
S1-RBD-36	RBD	—			—		
S1-RBD-37	RBD	—			3.60E+05	8.88E-04	2.47E-09
S1-RBD-38	RBD	—			1.12E+06	9.84E-04	8.79E-10
S1-RBD-39	RBD	—			4.92E+05	7.77E-05	1.58E-10
S1-RBD-40	RBD	—			7.47E+05	2.77E-05	3.71E-11
S1-RBD-41	RBD	—			4.37E+05	1.39E-04	3.17E-10
S1-RBD-43	RBD	—			6.21E+05	1.82E-04	2.92E-10
S1-RBD-44	RBD	—			1.91E+05	6.97E-05	3.65E-10
S1-RBD-45	RBD	—			4.43E+05	4.14E-05	9.30E-11

S1-RBD-46	RBD	–			4.69E+05	5.79E-04	1.23E-09
S1-RBD-47	RBD	–			2.11E+05	6.45E-04	3.06E-09
S1-RBD-48	RBD	–			1.05E+05	2.42E-04	2.30E-09
S1-RBD-49	RBD	3.24E+05	3.24E-04	1.00E-09	3.15E+05	5.34E-04	1.69E-09
S1-RBD-51	RBD	–			3.77E+06	2.01E-03	5.33E-10
S2-1	S2	–			–		
S2-2	S2	–			–		
S2-3	S2	–			–		
S2-4	S2	–			–		
S2-5	S2	–			–		
S2-6	S2	–			–		
S2-7	S2	–			–		
S2-9	S2	–			–		
S2-10	S2	–			–		
S2-11	S2	–			–		
S2-13	S2	–			–		
S2-14	S2	–			–		
S2-15	S2	–			–		
S2-18	S2	–			–		
S2-22	S2	–			–		
S2-26	S2	–			–		
S2-33	S2	–			–		
S2-35	S2	–			–		
S2-36	S2	–			–		
S2-39	S2	–			–		
S2-40	S2	–			–		
S2-42	S2	–			–		
S2-47	S2	–			–		
S2-57	S2	–			–		
S2-59	S2	–			–		
S2-62	S2	–			–		

^a Curves were fit to a heterogeneous ligand model. Respective K_{on} , K_{off} , and K_D values are shown for each component.

^b Curves were fit to two-state reaction model. Respective K_{on} , K_{off} , and K_D values are shown for each binding state.

5 ^c Two peaks were observed in the melting curve. T_m s for both are reported.

“_” = not determined

NA = no activity

Table 2 (continued)

ID	Epitope	S2			T_m (°C)	SARS-CoV-2 PSV IC50 (s.e.m) (nM)
		K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (M)		
S1-1	RBD	—	—	—	66.5	6.74 (1.03)
S1-2	non-RBD	—	—	—	66.5	NA
S1-3	non-RBD	—	—	—	64	1030 (666)
S1-4	RBD	—	—	—	66	41.5 (3.68)
S1-5	RBD	—	—	—	65.25	NA
S1-6	RBD	—	—	—	65	56.1 (20.7)
S1-7	non-RBD	—	—	—	60.5	NA
S1-9	non-RBD	—	—	—	47.5	NA
S1-10	non-RBD	—	—	—	64	NA
S1-11	non-RBD	—	—	—	60	NA
S1-12	RBD	—	—	—	68	NA
S1-14	RBD	—	—	—	57.5	135.8 (36.4)
S1-17	non-RBD	—	—	—	65	1271 (888)
S1-19	RBD	—	—	—	64.5	139 (9.58)
S1-20	RBD	—	—	—	69	51.8 (3.72)
S1-21	RBD	—	—	—	70.5	68.1
S1-23	RBD	—	—	—	64	5.7 (2.163)
S1-24	non-RBD	—	—	—	71.5	868
S1-25	non-RBD	—	—	—	58	NA

S1-27	RBD	—	54	19.5 (4.90)
S1-28	RBD	—	66	66.0 (10.9)
S1-29	RBD	—	61.5	NA
S1-30	non-RBD	—	57	330
S1-31	RBD	—	72	78.7 (3.48)
S1-32	non-RBD	—	79	NA
S1-35	RBD	—	70.5	12.5 (0.101)
S1-36	RBD	—	63	48.5 (21.1)
S1-37	RBD	—	65	7.54
S1-38	RBD	—	64	63.2
S1-39	RBD	—	55	111 (4.03)
S1-41	non-RBD	—	62.5	732
S1-46	RBD	—	68	312 (14.0)
S1-48	RBD	—	60.5	5.82 (0.832)
S1-49	non-RBD	—	49, 74 ^c	356 (56.9)
S1-50	non-RBD	—	66	24,303
S1-51	RBD	—	56	608
S1-52	RBD	—	57.5	3,634
S1-53	RBD	—	51.5	3,406
S1-54	RBD	—	69	148
S1-55	RBD	—	54.5	2,353
S1-56	RBD	—	54	NA
S1-58	non-RBD	—	53.5	146
S1-60	non-RBD	—	62	NA
S1-61	RBD	—	60	NA
S1-62	RBD	—	71.5	4.95
S1-63	RBD	—	65	NA
S1-64	non-RBD	—	—	28.1
S1-65	non-RBD	—	—	13.3

S1-66	non-RBD	–	–	NA
S1-RBD-3	RBD	–	72	384 (18.7)
S1-RBD-4	RBD	–	64.5	17.5 (1.98)
S1-RBD-5	RBD	–	64	34.5
S1-RBD-6	RBD	–	66.5	77.2 (21.8)
S1-RBD-9	RBD	–	69	522
S1-RBD-10	RBD	–	–	52.9
S1-RBD-11	RBD	–	65	13.5 (5.50)
S1-RBD-12	RBD	–	67	NA
S1-RBD-14	RBD	–	65	NA
S1-RBD-15	RBD	–	59.5, 80 ^c	5.29 (1.44)
S1-RBD-16	RBD	–	61	79.2 (4.23)
S1-RBD-18	RBD	–	69.5	67.2 (1.92)
S1-RBD-19	RBD	–	60	3,902
S1-RBD-20	RBD	–	49, 70 ^c	12.4 (1.06)
S1-RBD-21	RBD	–	48.5, 70.5 ^c	14.1
S1-RBD-22	RBD	–	57.5	100 (0.078)
S1-RBD-23	RBD	–	61	7.31 (0.418)
S1-RBD-24	RBD	–	46, 67 ^c	218
S1-RBD-25	RBD	–	–	NA
S1-RBD-26	RBD	–	66	241 (81.4)
S1-RBD-27	RBD	–	71	65.8
S1-RBD-28	RBD	–	64.5	32.7 (3.07)
S1-RBD-29	RBD	–	74	9.53 (1.04)
S1-RBD-30	RBD	–	65	25.0 (3.57)
S1-RBD-32	RBD	–	65	NA
S1-RBD-34	RBD	–	64	NA
S1-RBD-35	RBD	–	57, 68 ^c	12.3 (2.40)
S1-RBD-36	RBD	–	71	NA
S1-RBD-37	RBD	–	71	391

S1-RBD-38	RBD	–			68.5	84.6 (22.7)
S1-RBD-39	RBD	–			67.5	77.8
S1-RBD-40	RBD	–			70	25.6 (5.88)
S1-RBD-41	RBD	–			–	17.0
S1-RBD-43	RBD	–			68	33.6 (1.33)
S1-RBD-44	RBD	–			57.5	93.4
S1-RBD-45	RBD	–			53	22.6
S1-RBD-46	RBD	–			75.5	48.0
S1-RBD-47	RBD	–			53.5	127 (11.6)
S1-RBD-48	RBD	–			58, 63 ^c	54.5
S1-RBD-49	RBD	–			66.5	37.6
S1-RBD-51	RBD	–			52, 61 ^c	70.9 (29.3)
S2-1	S2	6.32E+04	3.79E-04	6.00E-09	65.5	NA
S2-2	S2	1.26E+06	9.35E-05	7.41E-11	64.5	4,460 (902)
S2-3	S2	2.62E+05	7.21E-05	2.76E-10	65	2,234 (751)
S2-4	S2	2.44E+06	2.62E-04	1.08E-10	56	NA
S2-5	S2	9.35E+05	2.74E-04	2.93E-10	66	NA
S2-6	S2	–			61.5	NA
S2-7	S2	1.66E+06	9.36E-05	5.62E-11	61	NA
S2-9	S2	9.29E+05	2.32E-04	2.50E-10	64.5	NA
S2-10	S2	9.31E+04	3.13E-04	3.37E-09	59, 64.5 ^c	1,718
S2-11	S2	7.94E+06	1.12E-03	1.41E-10	69.5	NA
S2-13	S2	7.02E+05	1.05E-04	1.49E-10	64.5	NA
S2-14	S2	3.16E+06	1.28E-03	4.07E-10	72.5	NA
S2-15	S2	–			70	NA
S2-18	S2	1.63E+06	4.87E-04	2.99E-10	47, 54.5 ^c	NA
S2-22	S2	–			–	NA
S2-26	S2	4.45E+05	8.15E-05	1.83E-10	76.5	NA
S2-33	S2	3.68E+05	5.58E-05	2.33E-10	70	NA
S2-35	S2	2.36E+05	4.72E-05	2.00E-10	77	NA
S2-36	S2	4.39E+06	3.69E-04	8.41E-11	74	NA

S2-39	S2	—			58	NA
S2-40	S2	5.08E+04	7.16E-05	1.41E-09	69.5	1,712 (828)
S2-42	S2	5.12E+05	3.77E-06	7.36E-12	69	NA
S2-47	S2	3.86E+05	1.14E-04	2.96E-10	40, 65 ^c	NA
S2-57	S2	2.33E+06	7.18E-04	3.08E-10	67	NA
S2-59	S2	—			37.5, 60 ^c	NA
S2-62	S2	1.65E+06	1.12E-04	6.81E-11	64, 77.5 ^c	12,979
CoV2-YD-1	S1 non-RBD	—			—	
CoV2-YD-2	S1 non-RBD	—			—	
CoV2-YD-3	S1 non-RBD	—			—	
CoV2-YD-4	S1 non-RBD	—			—	
CoV2-YD-5	RBD	—			—	
CoV2-YD-6	RBD	—			—	
CoV2-YD-7	RBD	—			—	
CoV2-YD-8	RBD	—			—	
CoV2-YD-9	RBD	—			—	
CoV2-YD-10	RBD	—			—	
CoV2-YD-11	RBD	—			—	
CoV2-YD-12	RBD	—			—	
CoV2-YD-13	RBD	—			—	
CoV2-YD-14	RBD	—			—	
CoV2-YD-15	S2	—			—	
CoV2-YD-16	S2	—			—	
CoV2-YD-17	S2	—			—	
CoV2-YD-18	S2	—			—	
CoV2-YD-19	S2	—			—	

CoV2-YD-20	S2	—	—
CoV2-YD-21	S2	—	—
CoV2-YD-22	S2	—	—
CoV2-YD-23	S2	—	—
CoV2-YD-24	S2	—	—
CoV2-YD-25	S2	—	—
CoV2-YD-26	S2	—	—
CoV2-YD-27	S2	—	—
CoV2-YD-28	S2	—	—
CoV2-YD-29	S2	—	—
CoV2-YD-30	S2	—	—
CoV2-YD-31	S1 non- RBD	—	—
CoV2-YD-32	S1 non- RBD	—	—
CoV2-YD-33	RBD	—	—
CoV2-YD-34	RBD	—	—
CoV2-YD-35	RBD	—	—
CoV2-YD-36	RBD	—	—
CoV2-YD-37	RBD	—	—
CoV2-YD-38	RBD	—	—
Rescue-1	RBD	—	—
Rescue-2	RBD	—	—
Rescue-3	RBD	—	—

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While illustrative examples have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

1. A VHH chain of an anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nanobody, wherein the amino acid sequence of the VHH chain comprises any one of the sequences as set forth in SEQ ID NOs: 117 – 153.
- 5 2. The VHH chain of claim 1, wherein SEQ ID NOs: 121-130, and 150-157 are selective for binding to Receptor Binding Domain (RBD) of SARS-CoV-2 spike protein.
3. The VHH chain of claim 1, wherein SEQ ID NOs: 117-120, 147, and 148 are selective for binding to non-Receptor Binding Domain (RBD) of SARS-CoV-2 spike protein.
4. The VHH chain of claim 1, wherein SEQ ID NOs: 131-146 are selective for
10 binding to S2 domain of the SARS-CoV-2 spike protein.
5. An immunoconjugate comprising:
 - (a) a VHH chain comprising a sequence selected from SEQ ID NOs: 117 – 153; and
 - (b) a conjugating part comprising a detectable marker, a drug, a radionuclide, or an enzyme.
- 15 6. A pharmaceutical composition comprising:
 - (a) a VHH chain comprising a sequence selected from SEQ ID NOs: 117 – 153; and
 - (b) a pharmaceutically acceptable carrier.
7. A method comprising administering to an individual who has, is suspected of having, or is at risk of contracting a SARS-CoV-2 infection, a composition comprising a VHH
20 chain of an anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, wherein the amino acid sequence of the VHH chain comprises any one of the sequences as set forth in SEQ ID NOs: 117 – 153.
8. The method of claim 7, wherein the individual has the SARS-CoV-2 infection.
9. The method of claim 8, wherein the individual has been diagnosed with
25 COVID-19.
10. A polynucleotide encoding a VHH chain of an anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), wherein the amino acid sequence of the VHH chain comprises any one of the sequences as set forth in SEQ ID NOs: 117 – 153.

11. The polynucleotide of claim 10, wherein the polynucleotide is present in an expression vector.

12. A method comprising allowing expression of the expression vector of claim 11 by cells in a cell culture, and separating from the cell culture a VHH chain of an anti-Severe
5 Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

13. A polynucleotide that selectively hybridizes to a polynucleotide encoding an amino acid of any one of SEQ ID NOs: 117 – 153.

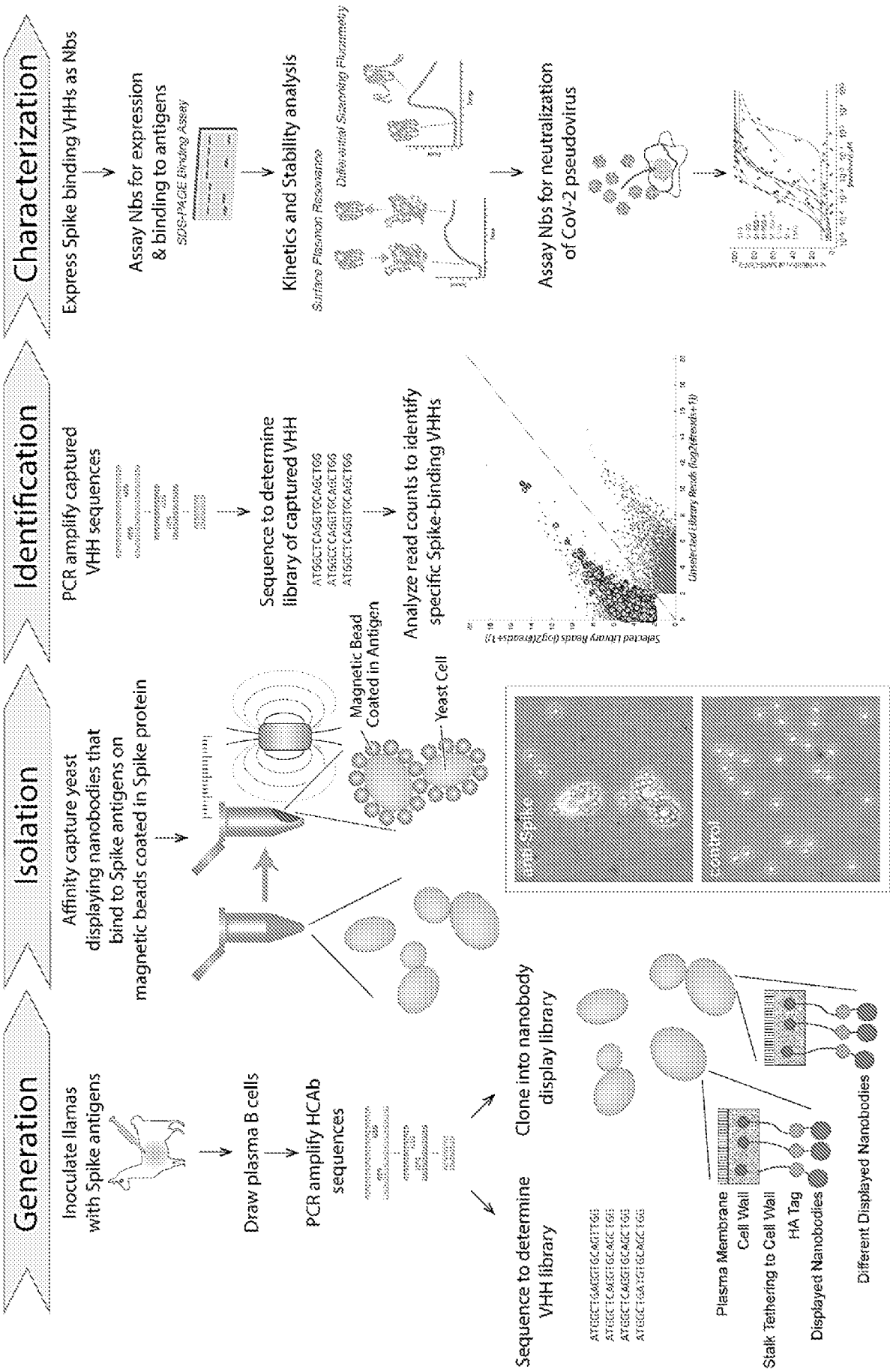


Figure 1

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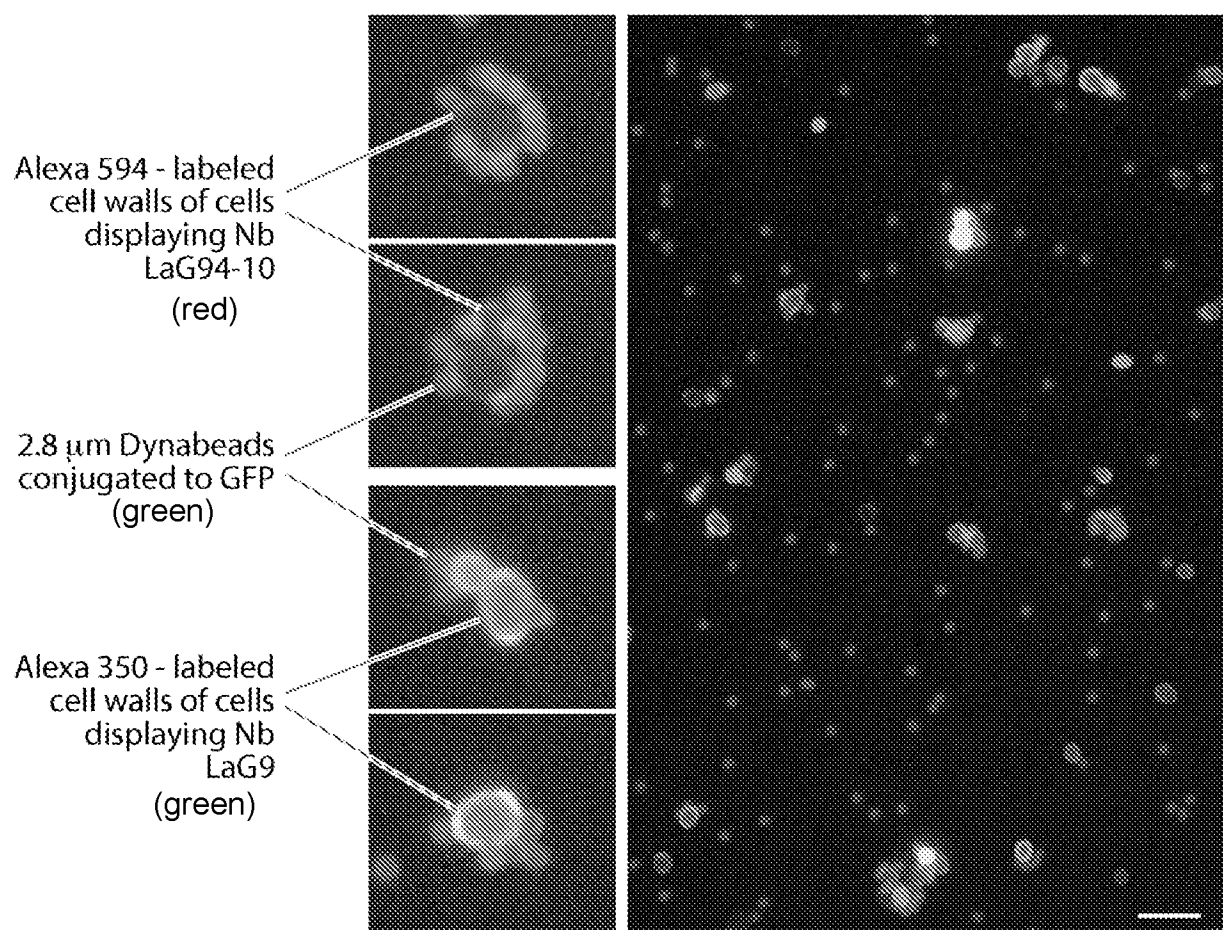


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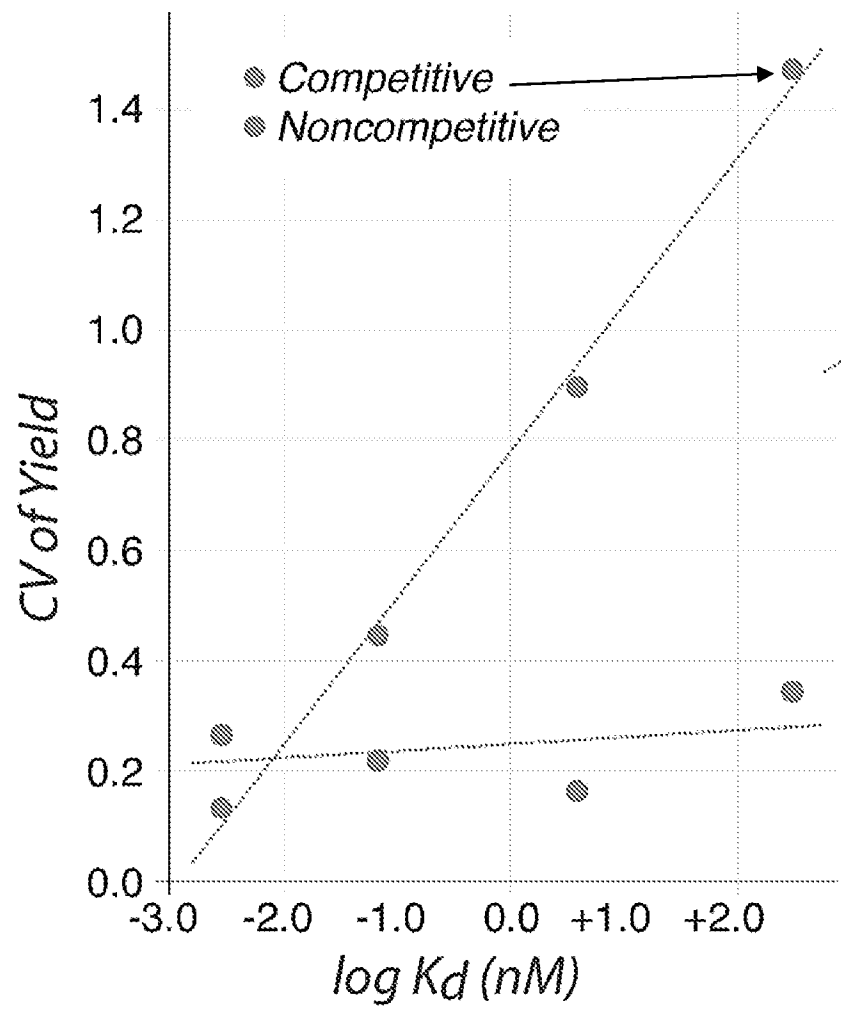


Figure 2B

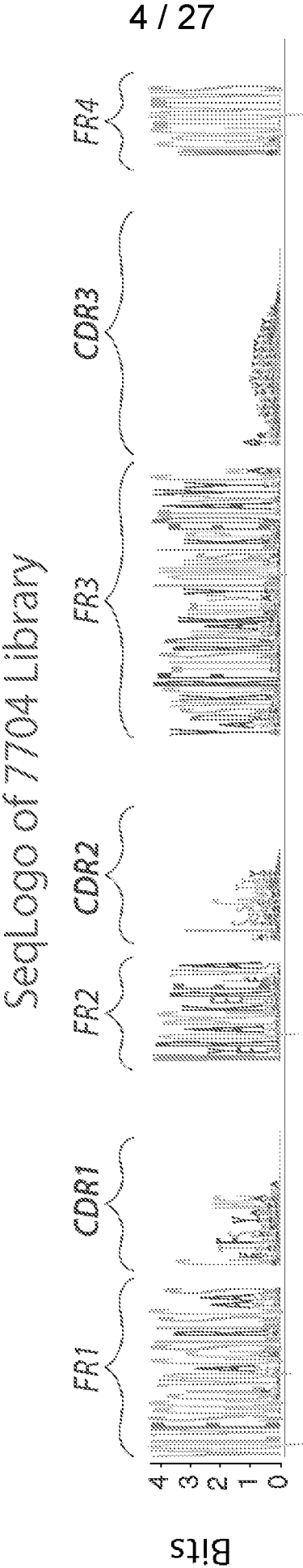


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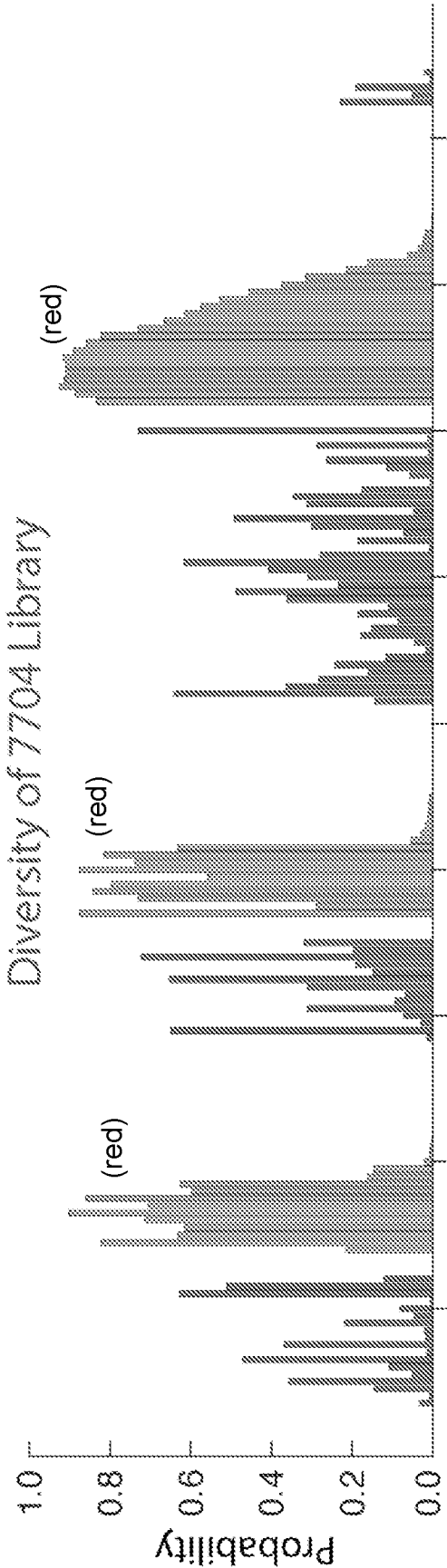


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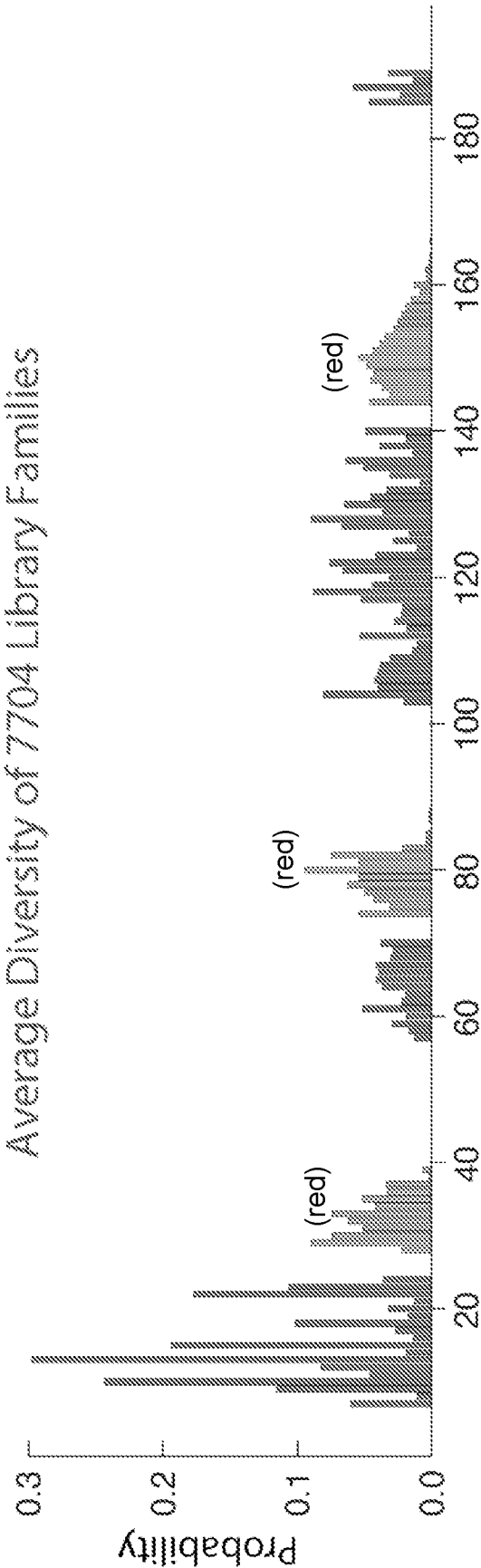


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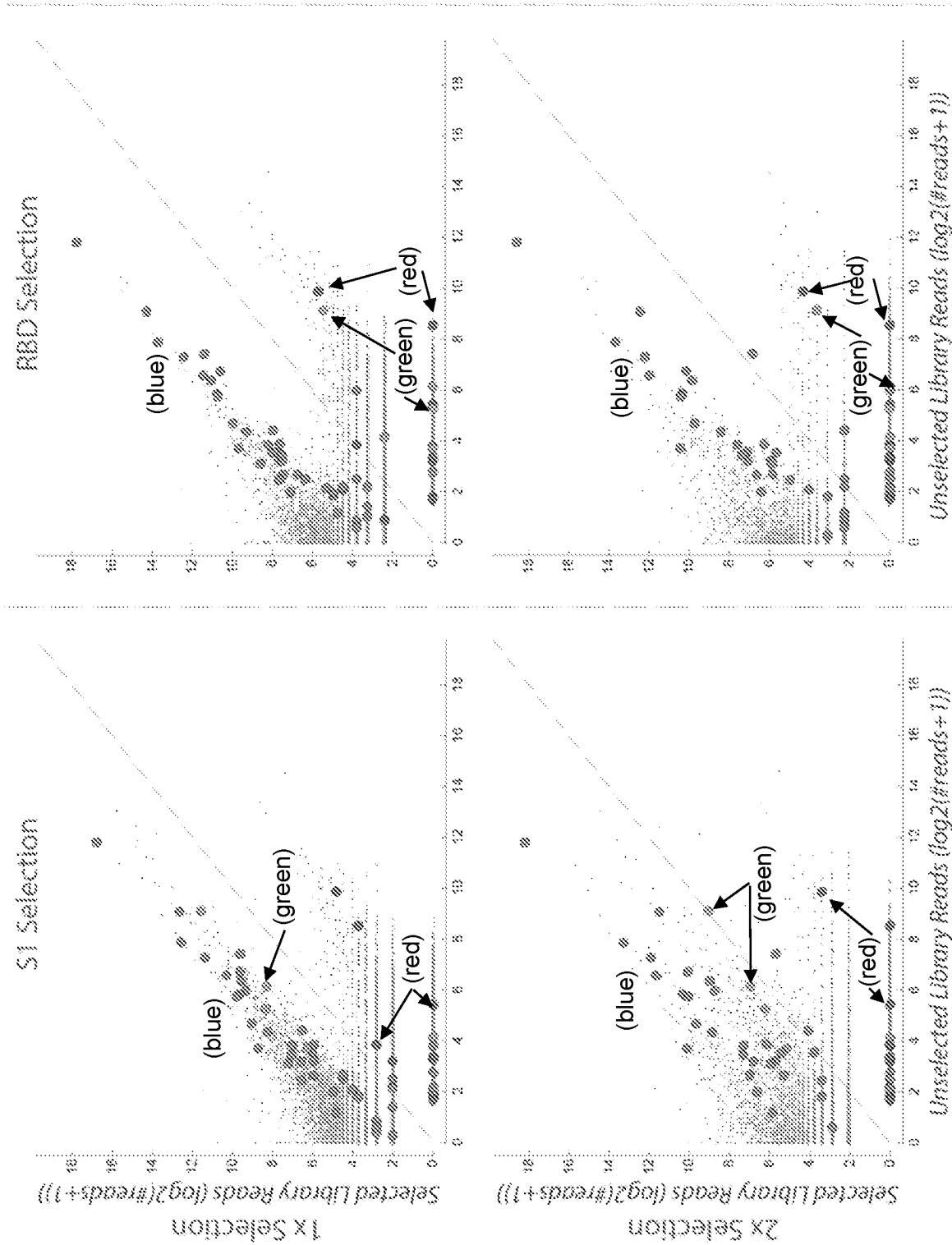


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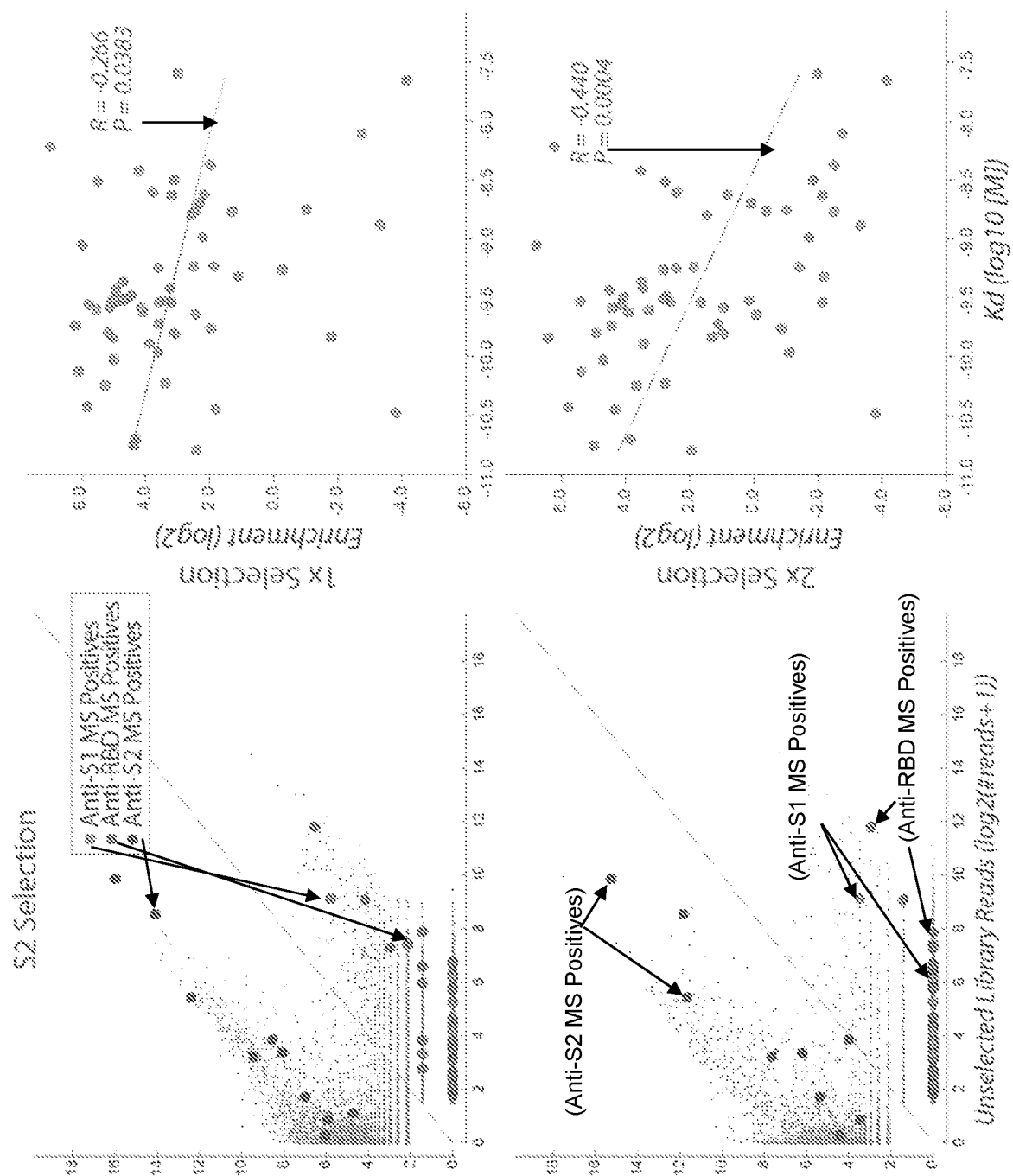


Figure 4B

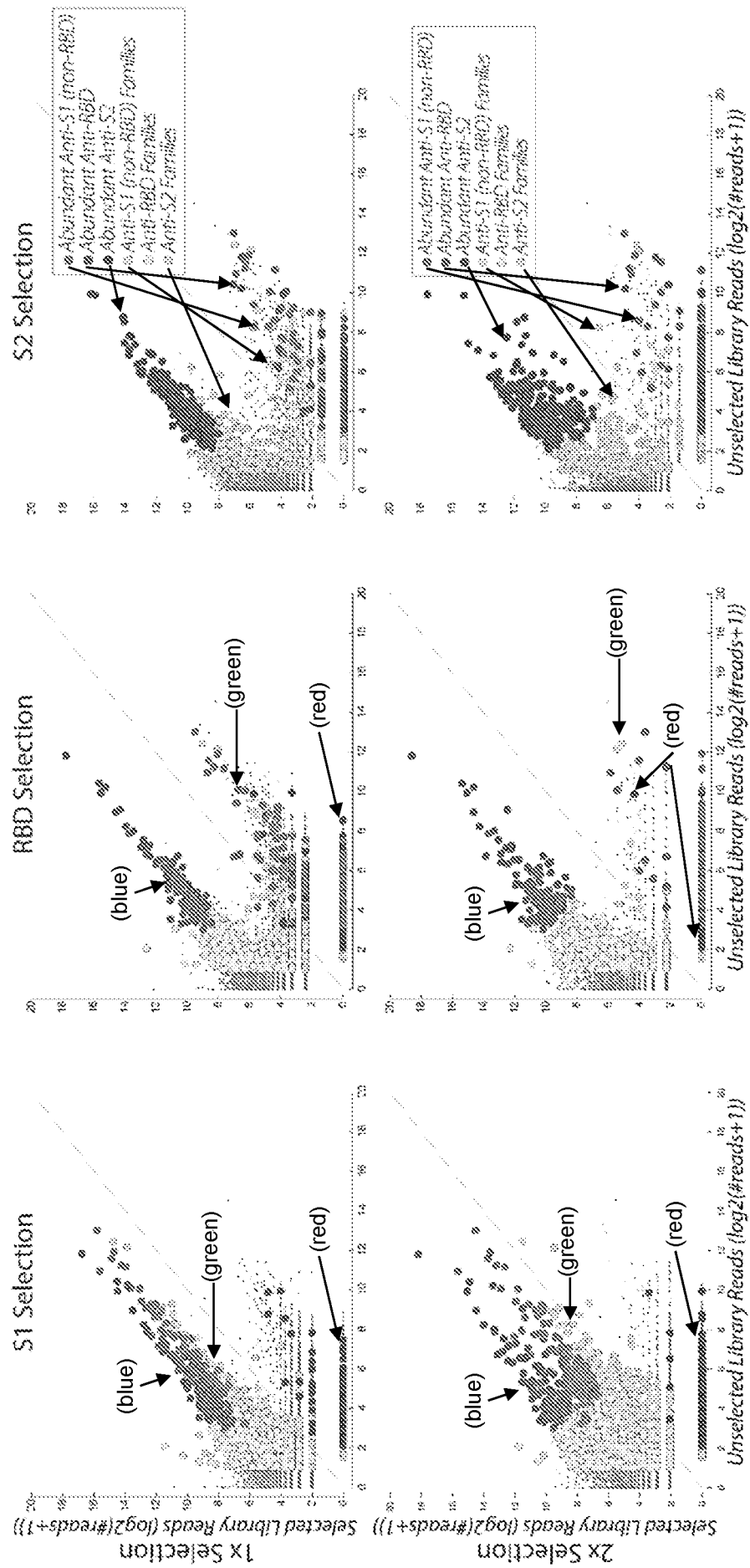


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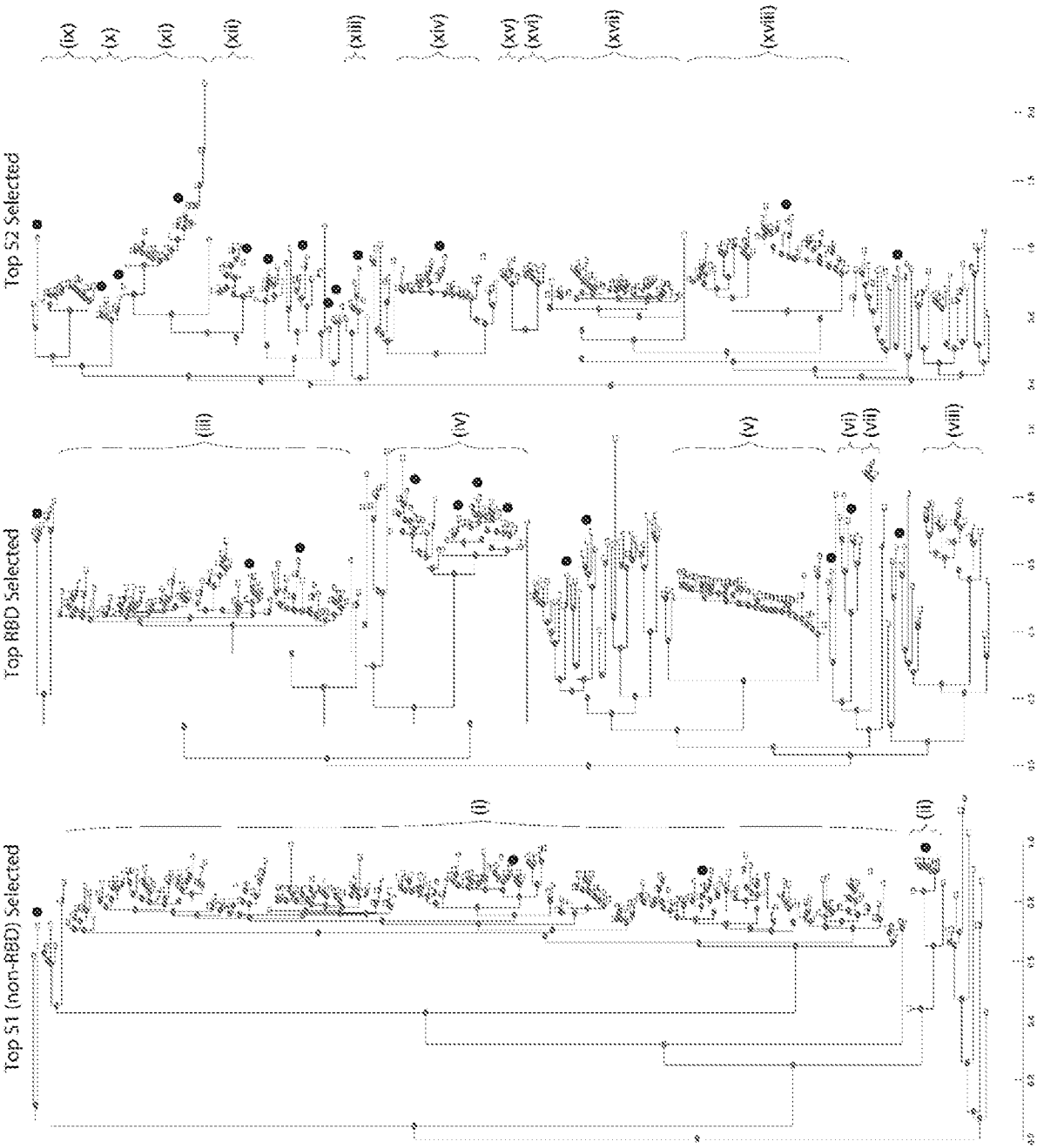


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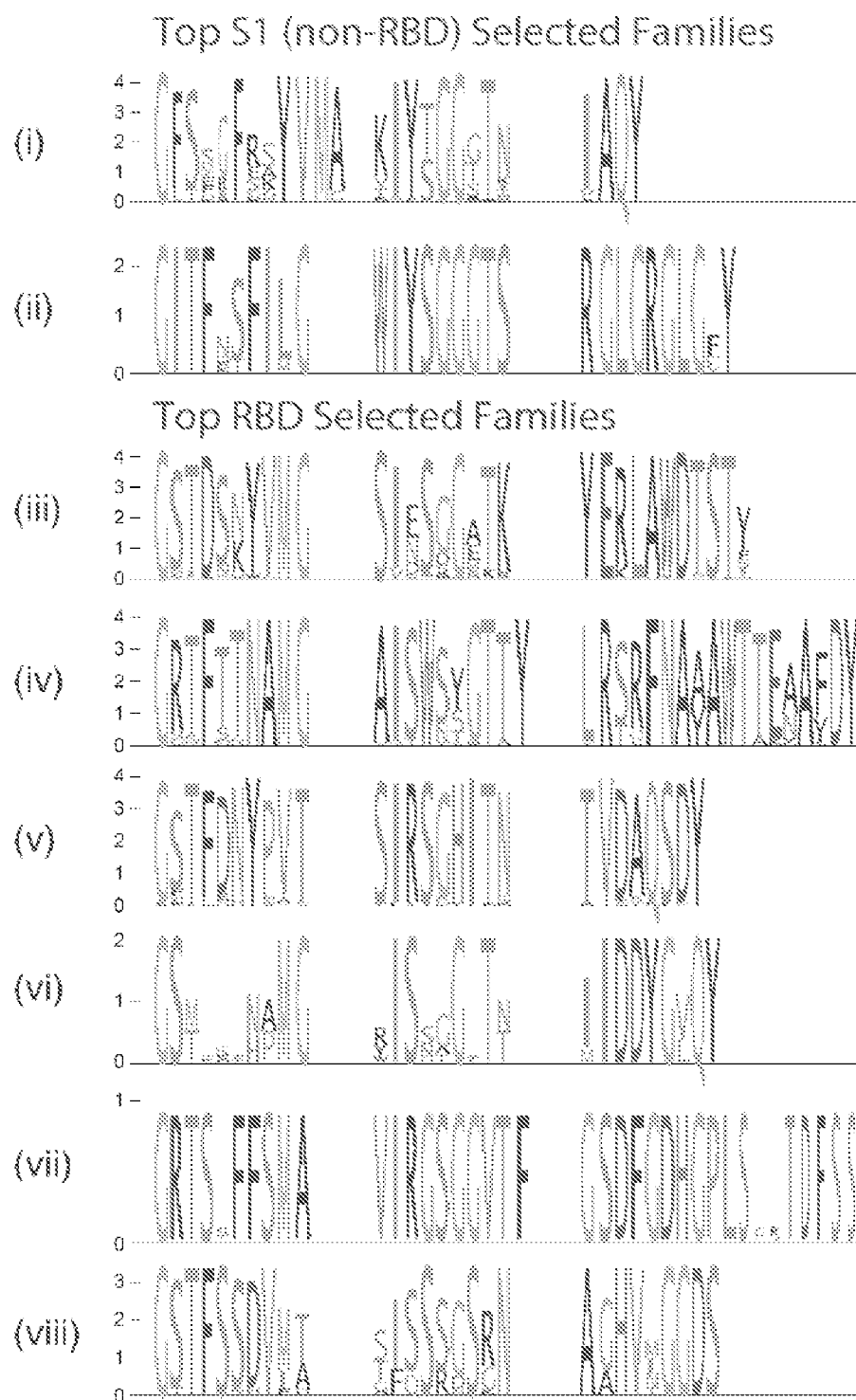


Figure 5C

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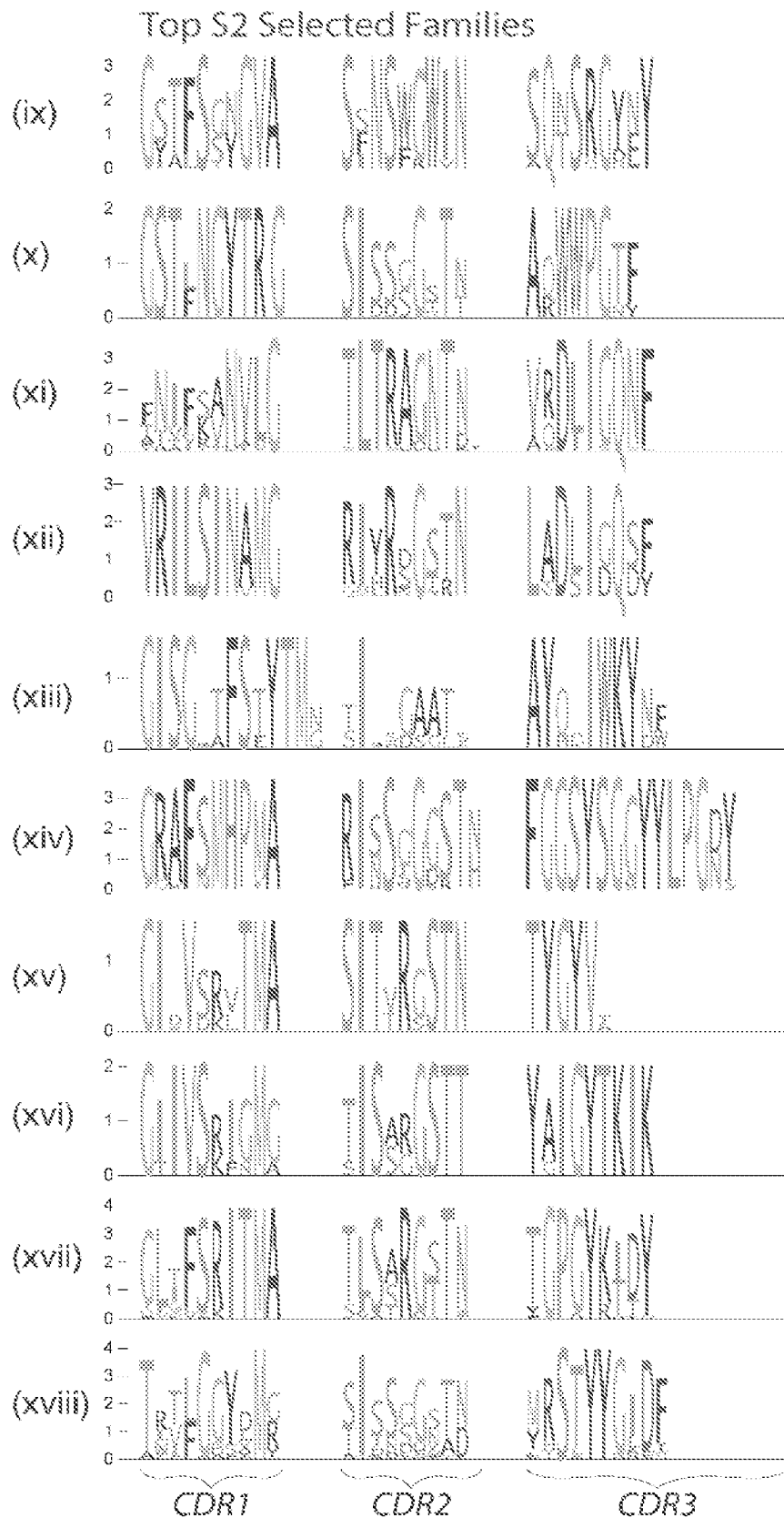


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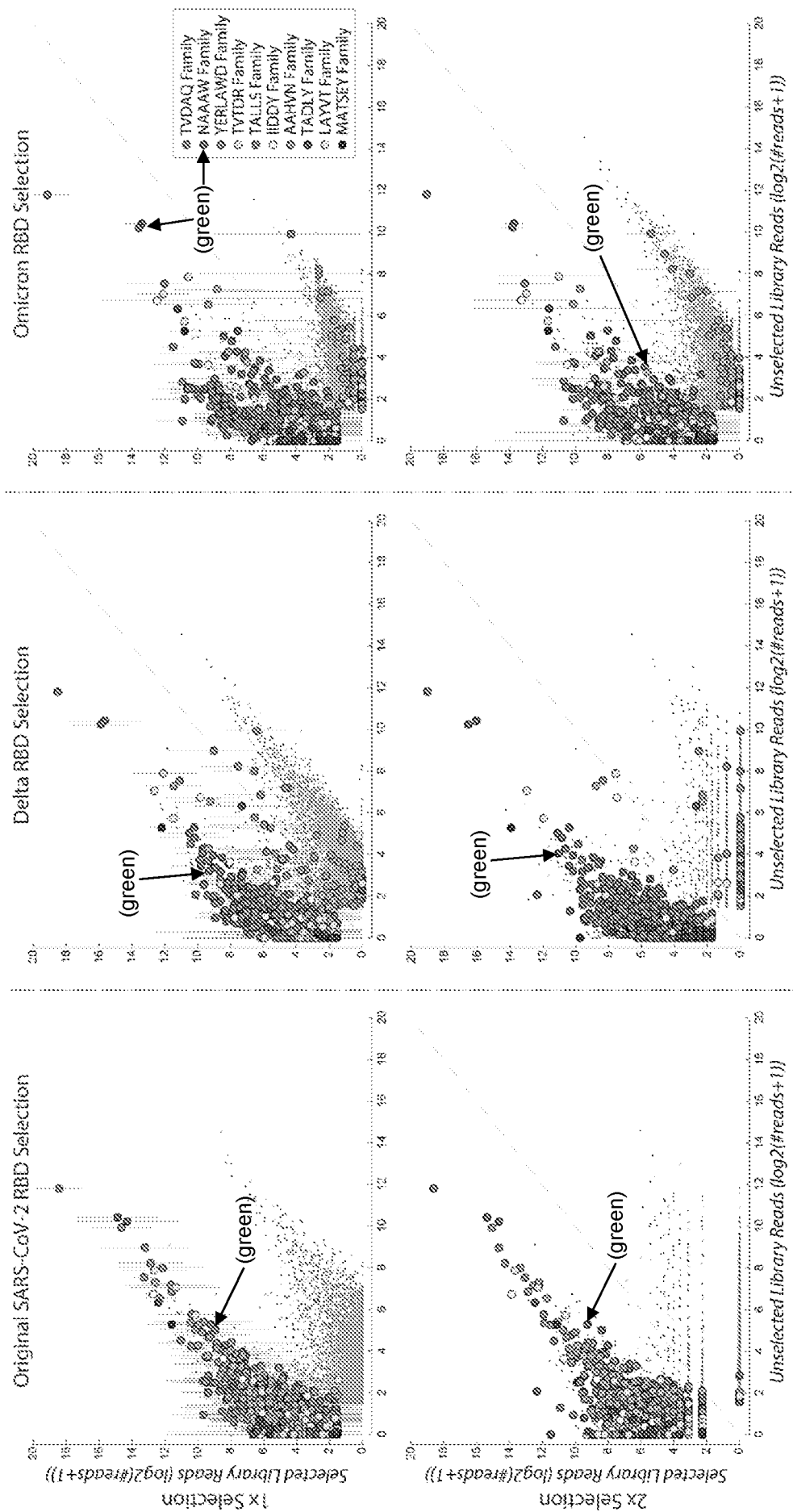


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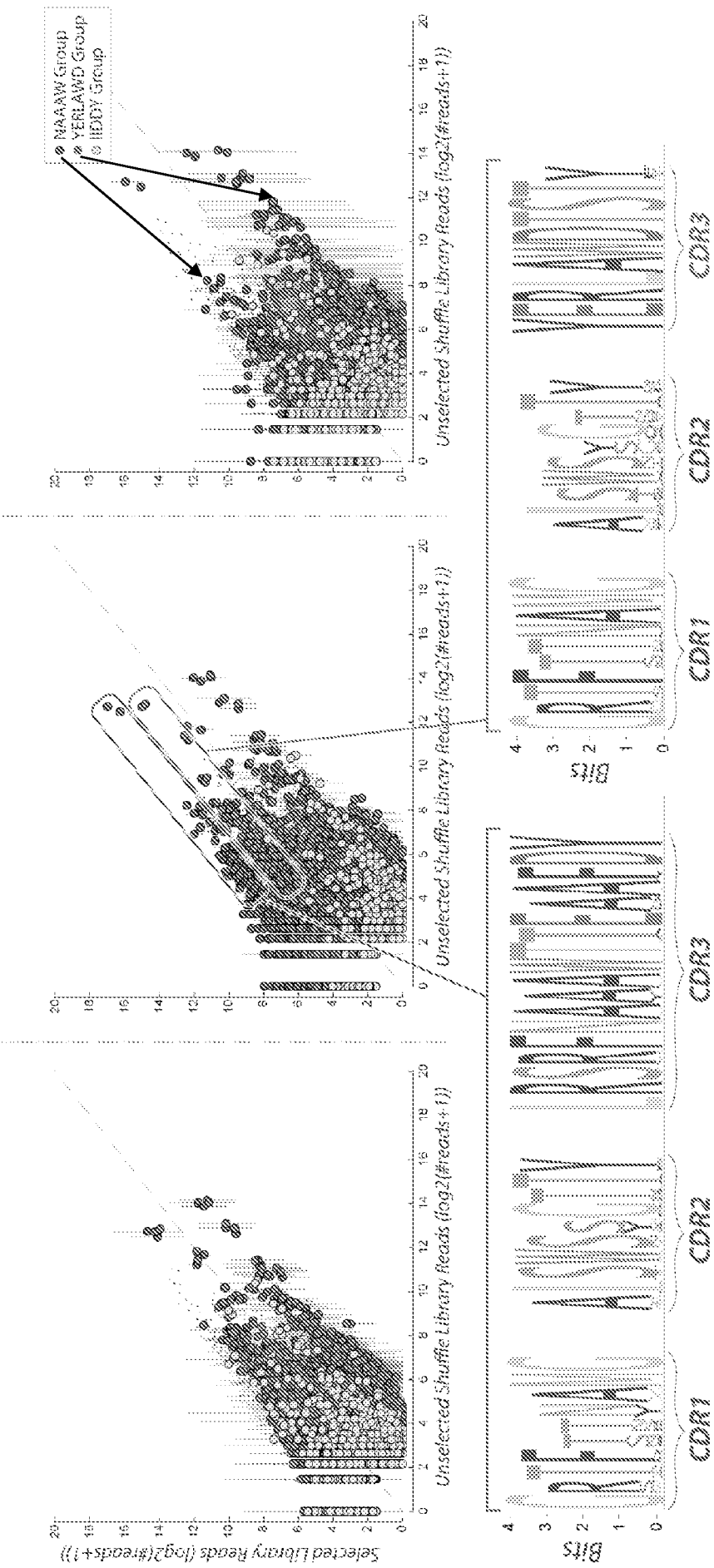


Figure 6B

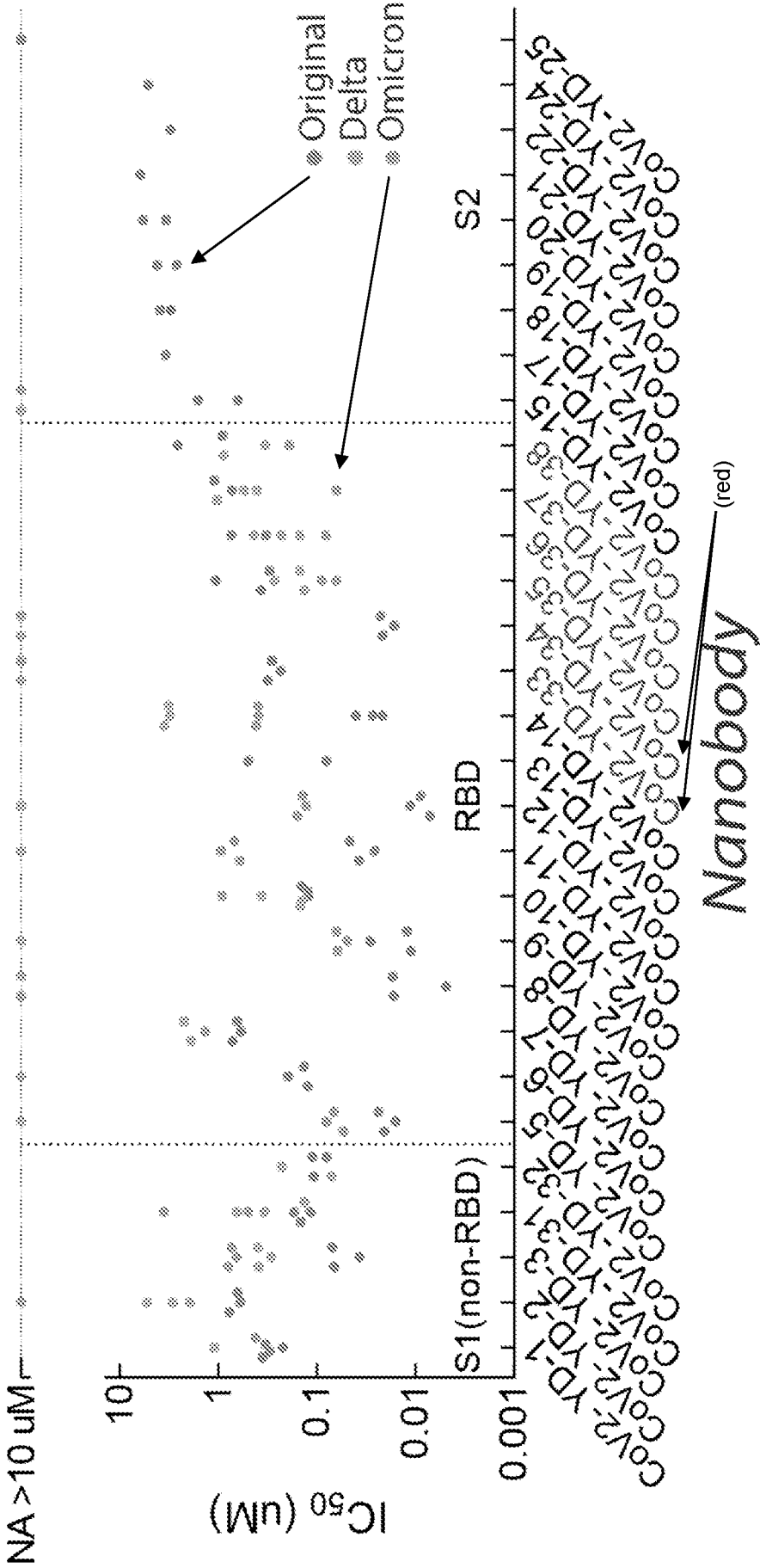


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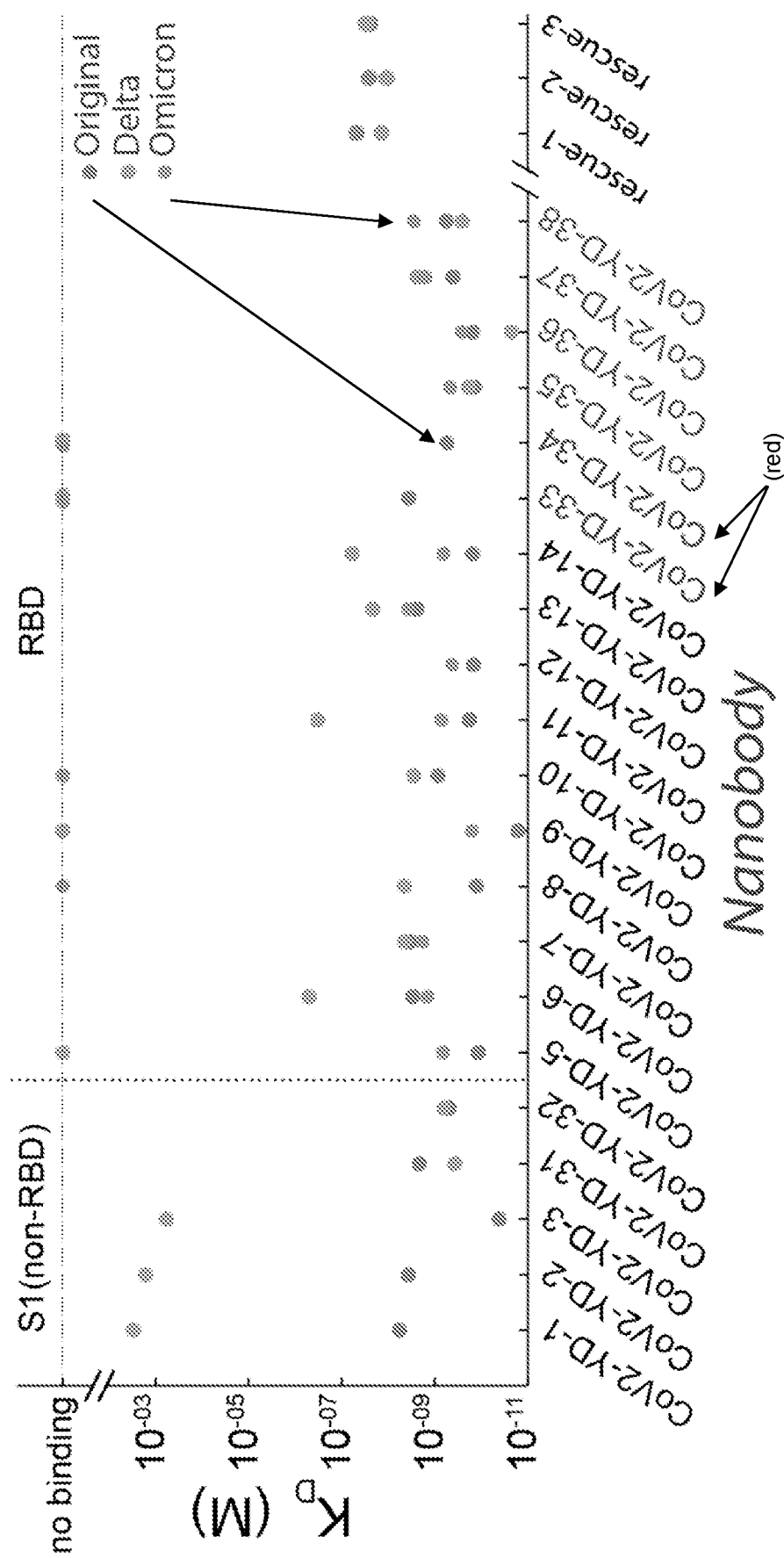


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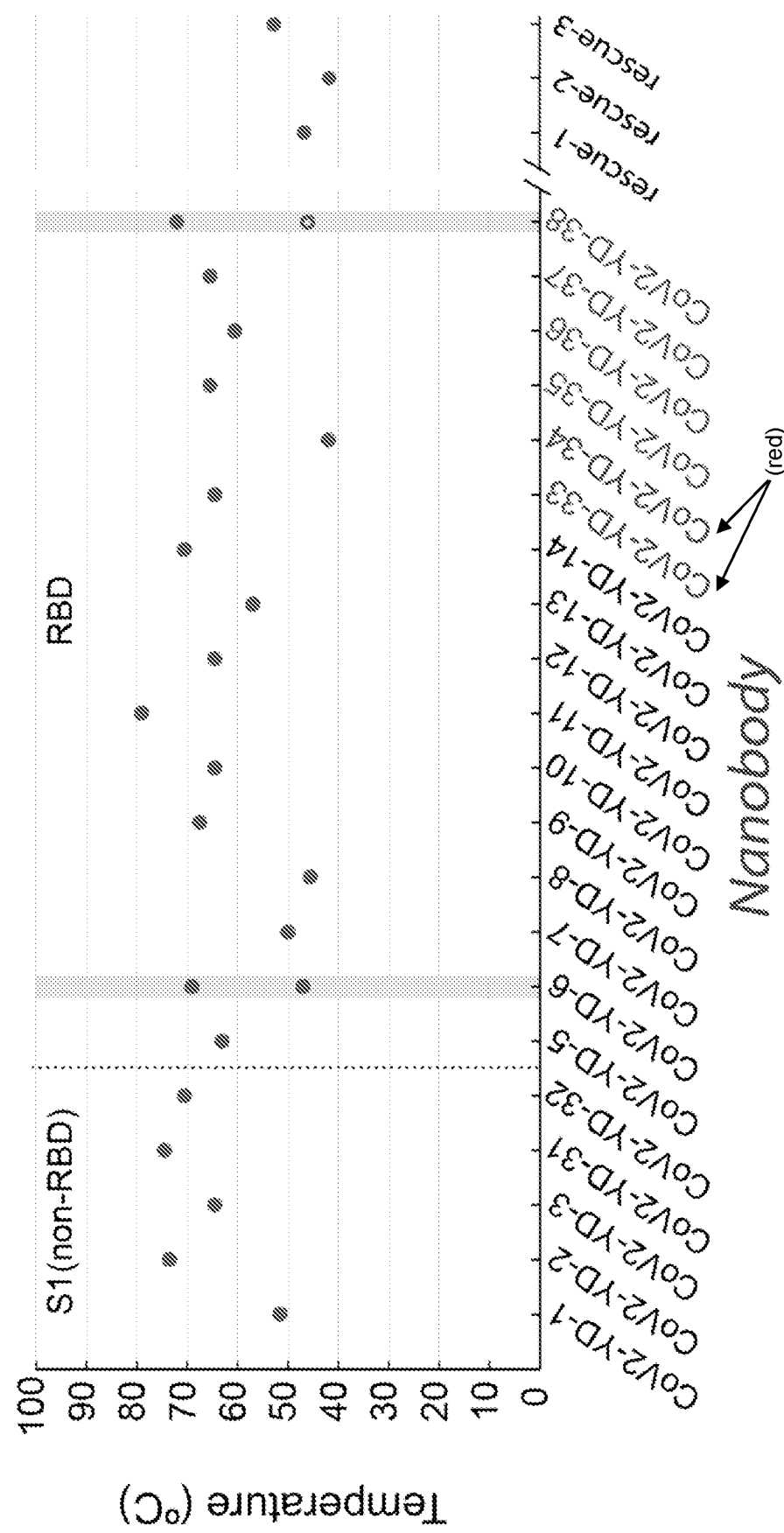


Figure 7C

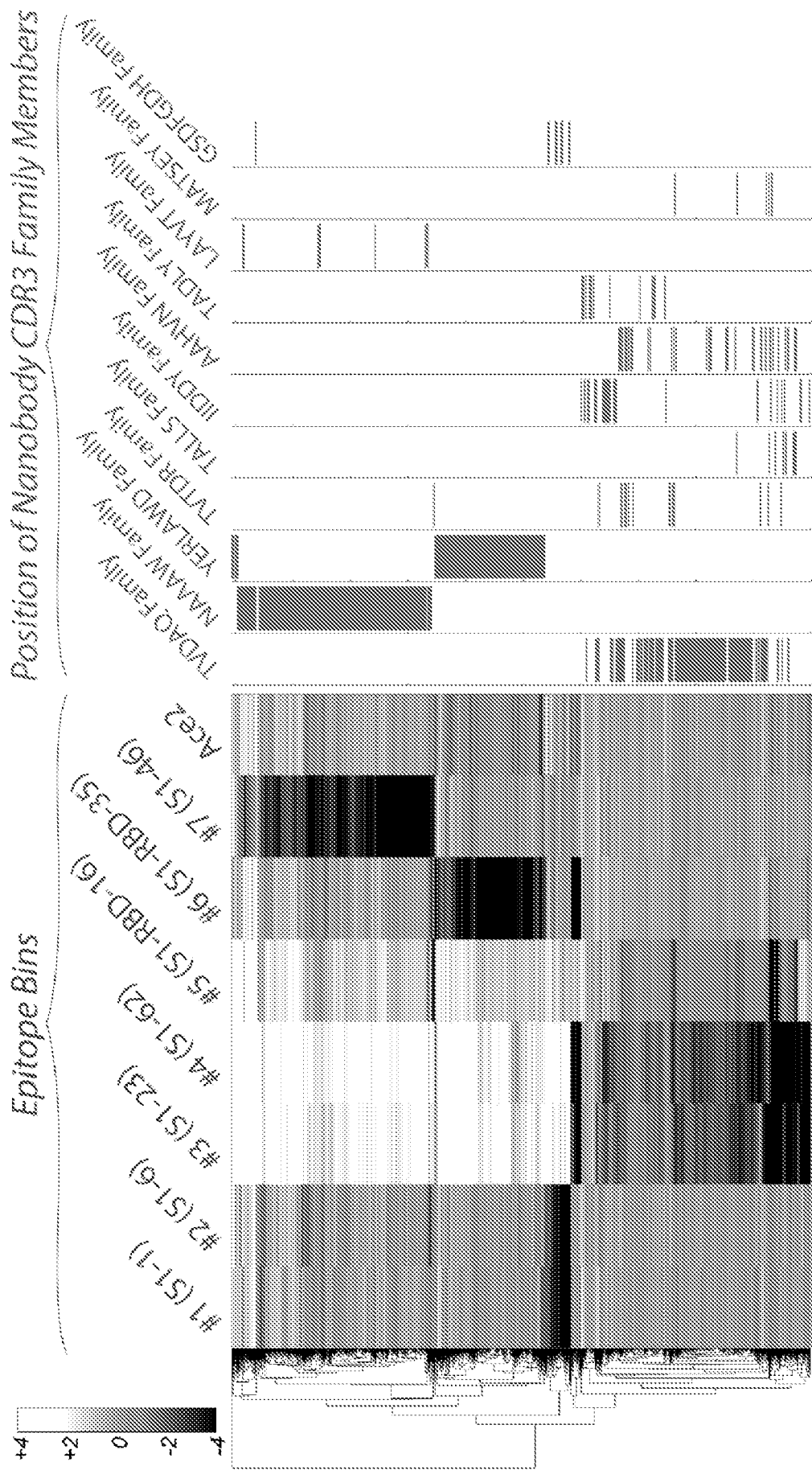


Figure 8

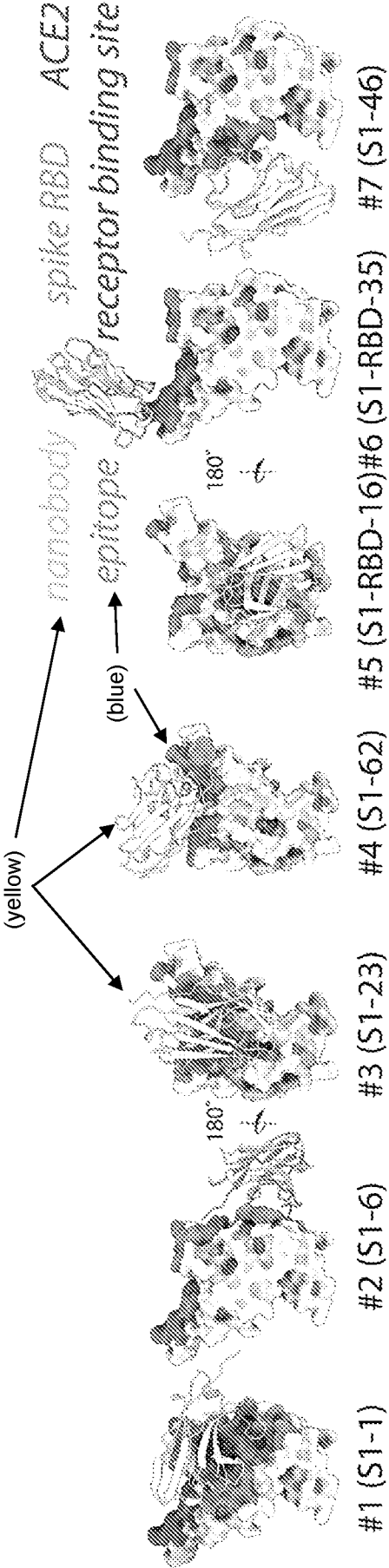


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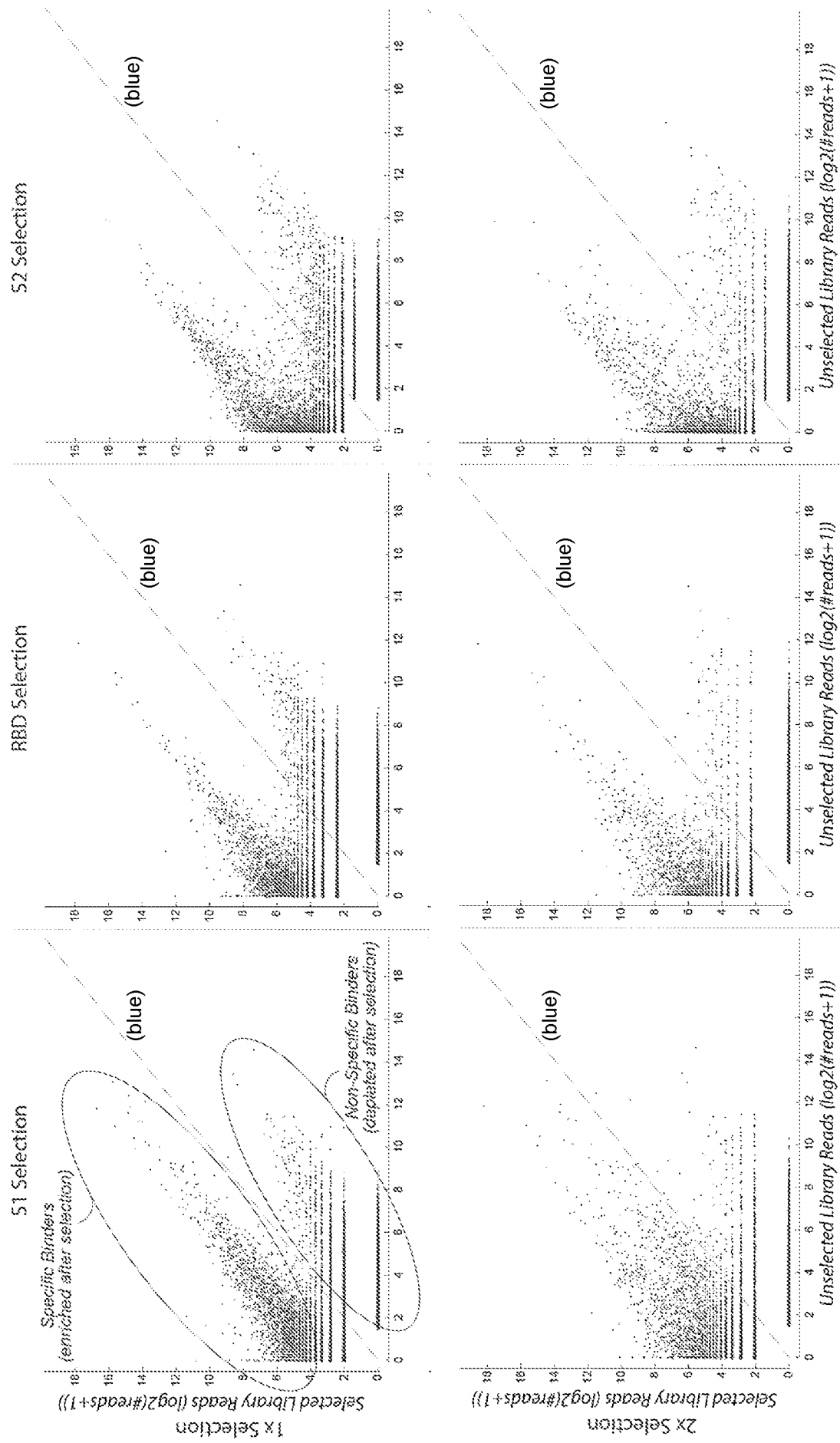


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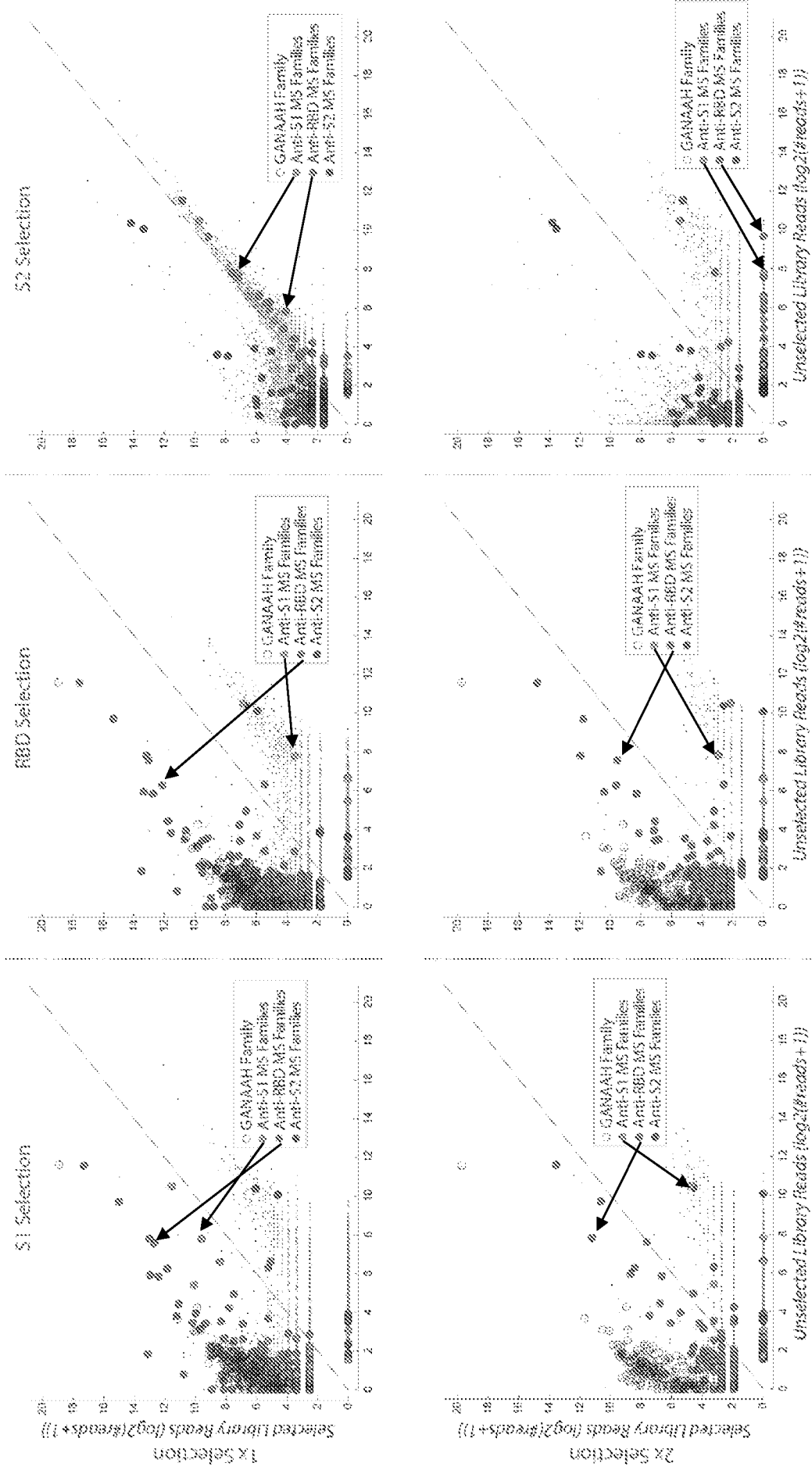


Figure 10

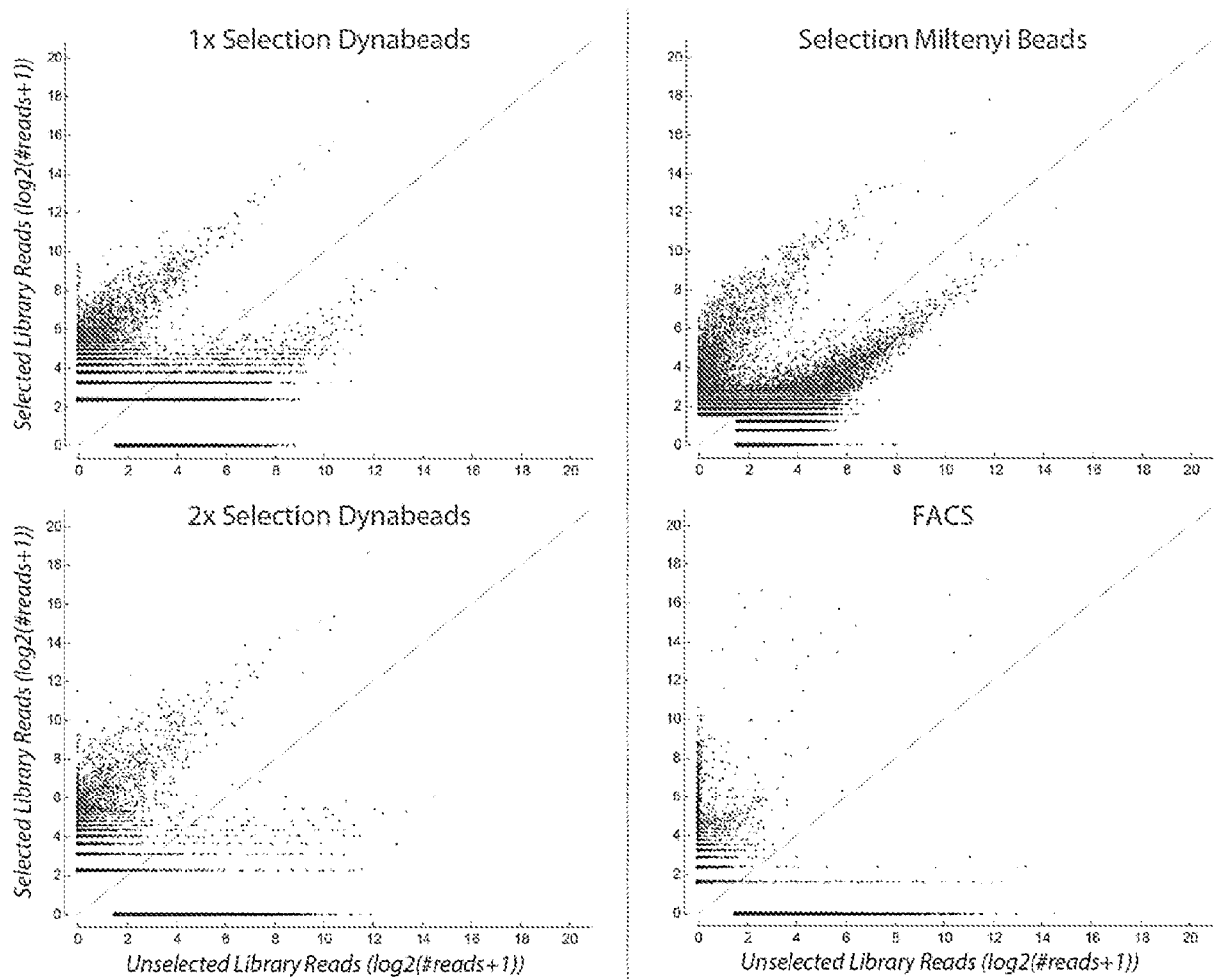


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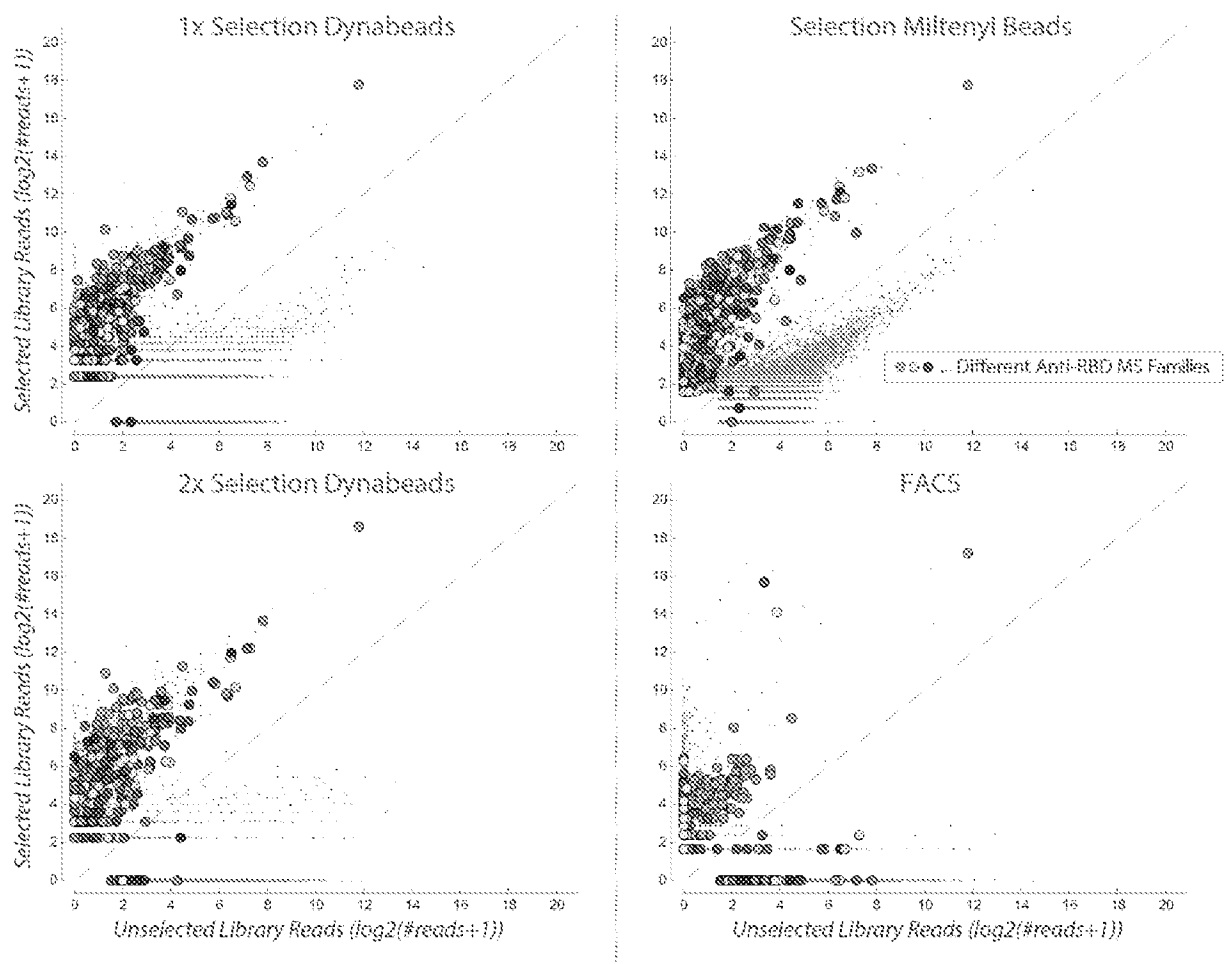


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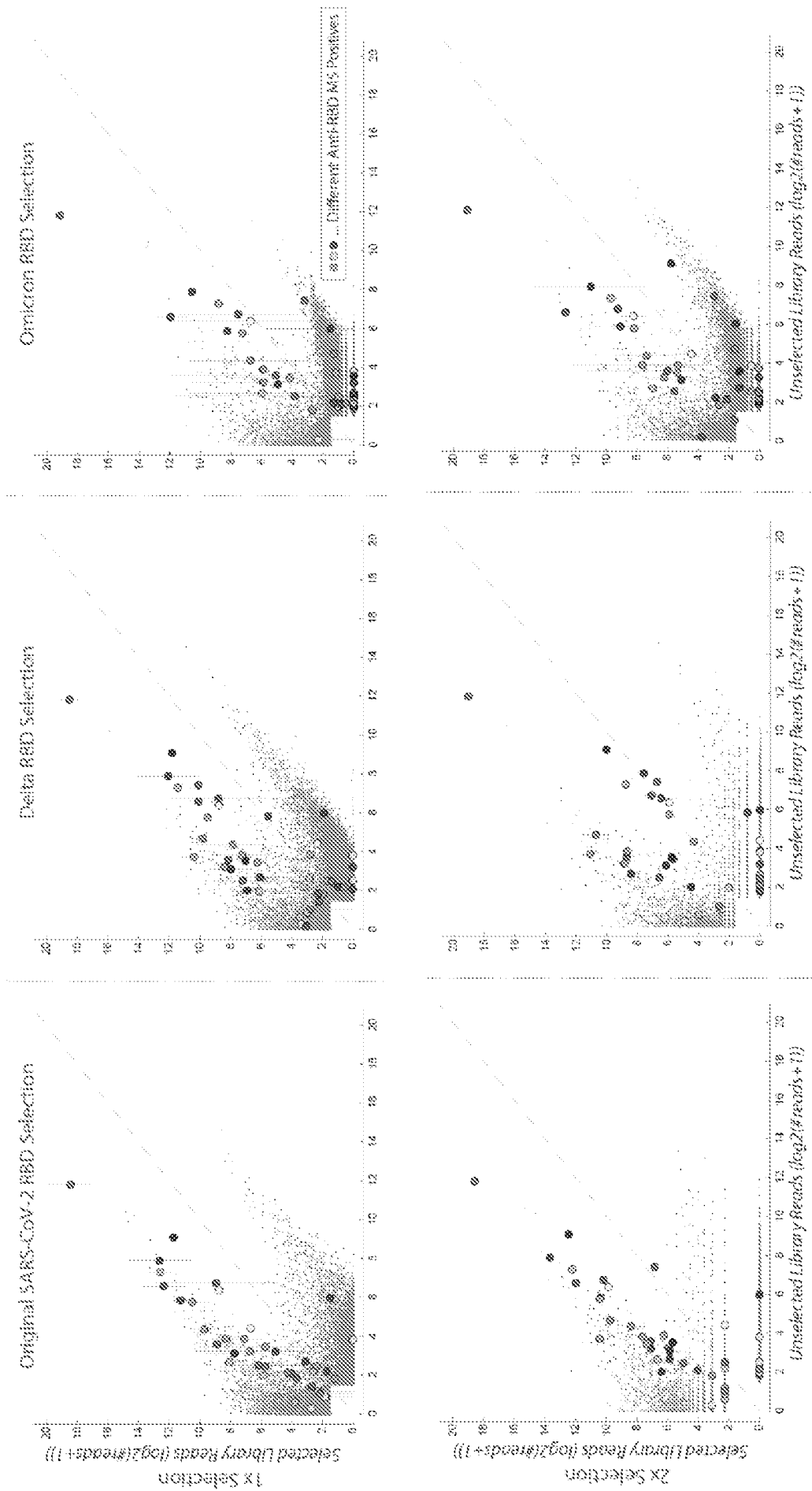


Figure 12

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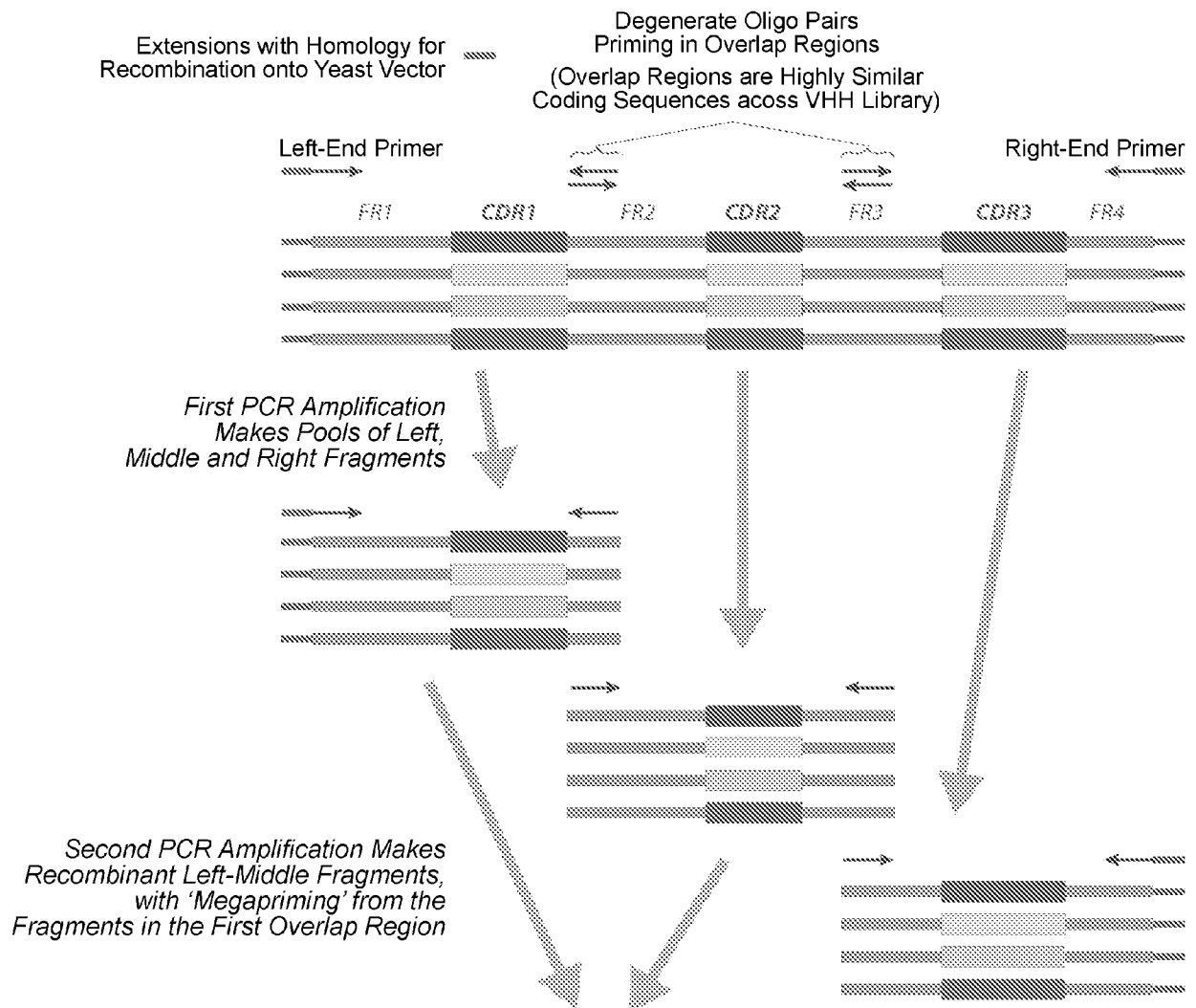


Figure 13

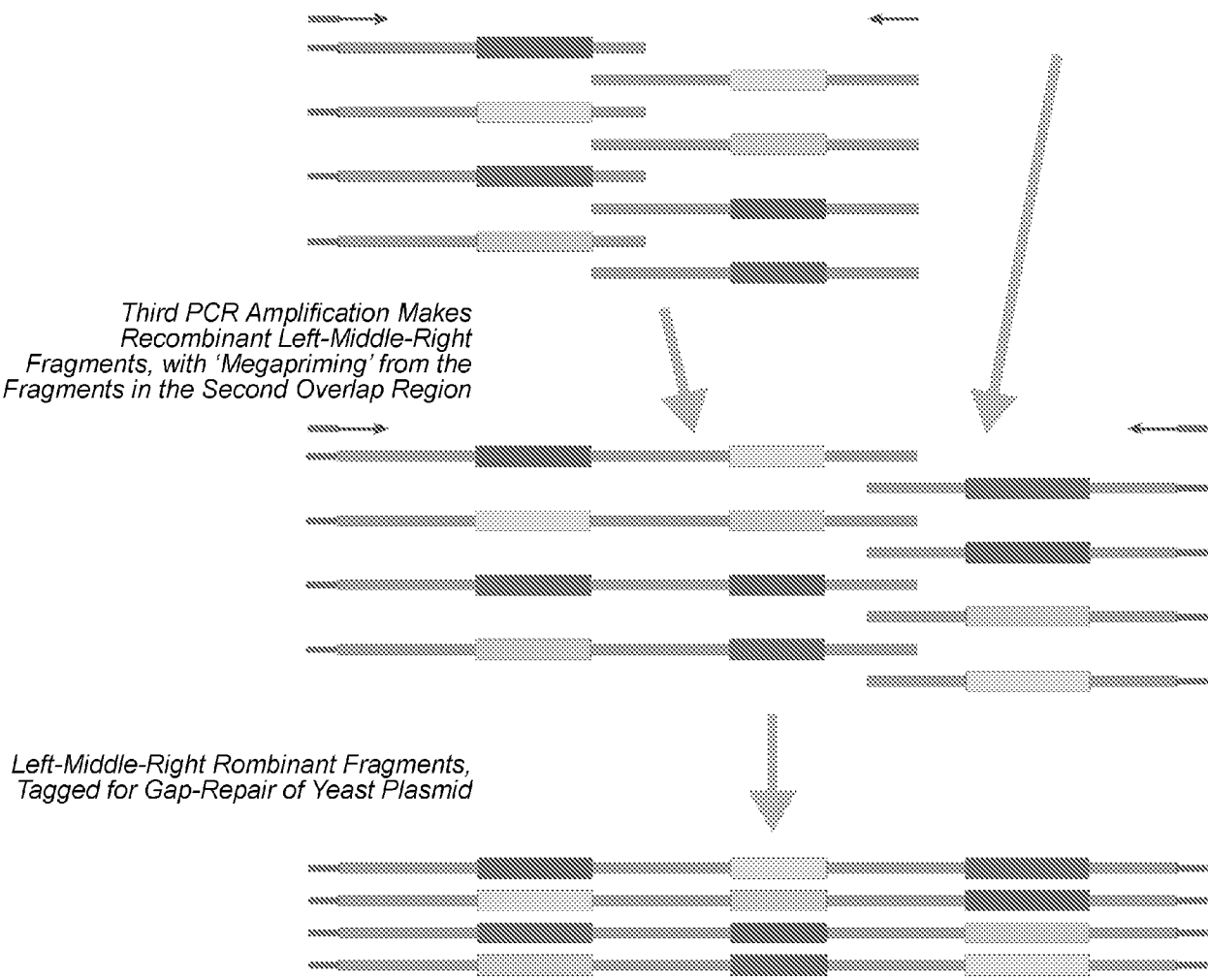


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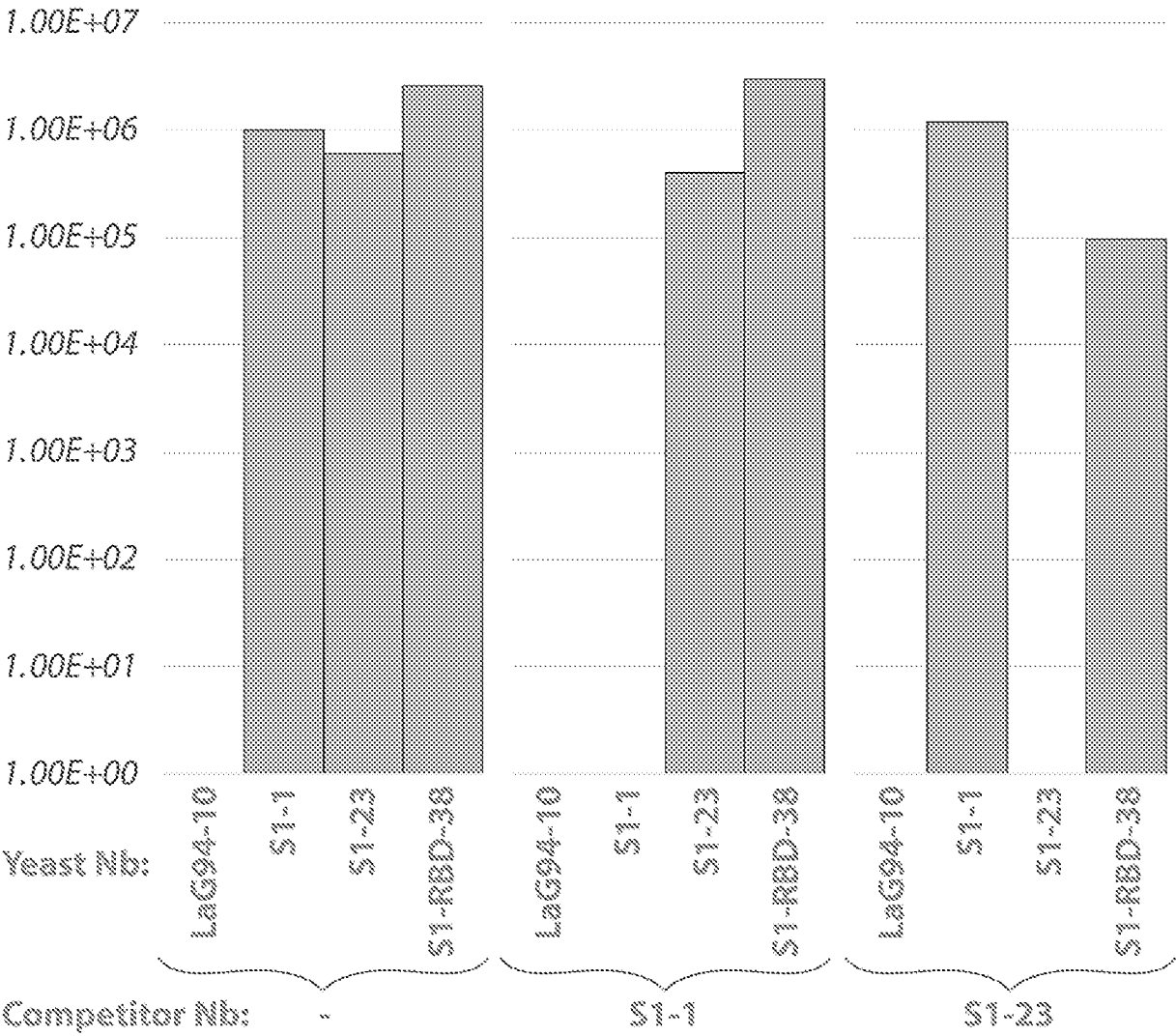


Figure 14

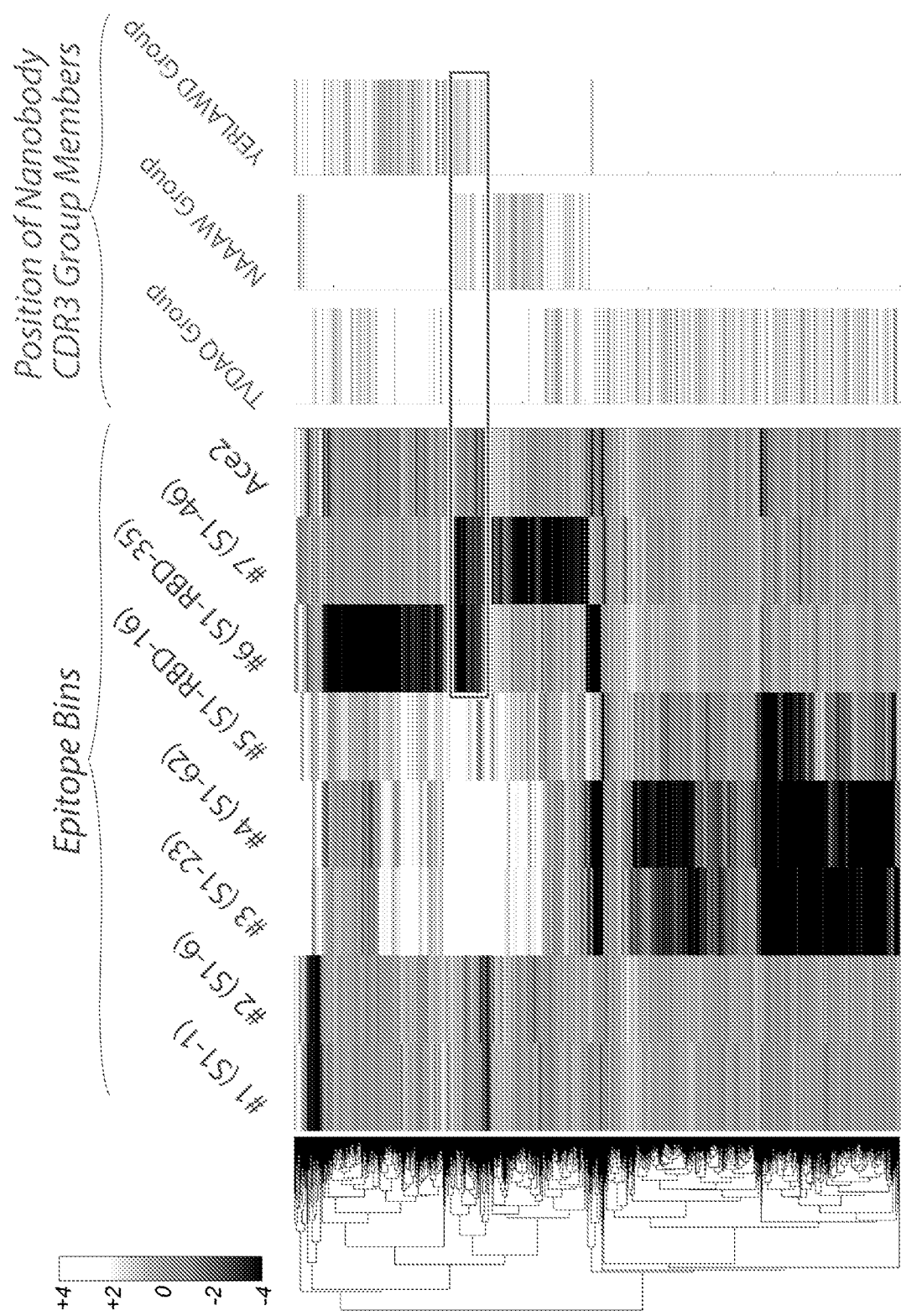


Figure 15